

MAR 2 1927

VOLUME 3

NUMBER 3

ARCHIVES OF PATHOLOGY AND LABORATORY MEDICINE

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MARCH, 1927

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH
DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, \$5.00

Entered as Second-Class Matter, Jan. 26, 1926, at the Postoffice at Chicago, Illinois, Under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in the Act of Feb. 26, 1925, authorized Jan. 25, 1926.

NUMBER

Vol. 1, No. 1, 1911

The Journal of the

Philosophical Society

of the University of

Chicago

Published by the

University of Chicago

Press

Chicago, Ill.

1911

Price, \$1.00

Per Annum, \$3.00

Single Copies, 50c

Subscription, \$10.00

Foreign, \$12.00

Postage, 50c

Advertising, \$5.00

Editorial, \$1.00

Manuscript, \$1.00

Proofs, \$1.00

Reprints, \$1.00

Index, \$1.00

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DEGENERATIVE CHANGES IN THE SKELETAL MUSCLES, PARTICULARLY IN INFEC- TIOUS DISEASES *

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STOCKHOLM, SWEDEN

The changes in the skeletal muscles that I will discuss chiefly are waxy and fatty degeneration.

WAXY DEGENERATION

Waxy degeneration was described, although incompletely, as early as 1841 by Bowmann, 1843 by Vogel and 1849 by Bennett. Rokitsky also described a muscular degeneration, probably of waxy nature, called by him colloidal metamorphosis. It was first through Zenker's¹ classic work (1864) on waxy degeneration in typhoid fever that it became generally known. Zenker considered the degeneration due to an invasion of albuminous substances from without. These substances were then supposed either to be broken up into granules (granular degeneration) within the muscle fiber or, after complete penetration of this, to cause it to swell (waxy degeneration).

Waldeyer and Erbst considered waxy degeneration a coagulation of muscle protein, especially myosin.

Beneke² emphasized the fact that all muscles coagulate in rigor mortis. He saw the explanation in a separation of the proteins from the muscles.

Nesti considered the degeneration to be due to injury to the contractile substance in muscle fibers lying between healthy fibers and the typical degeneration after partial absorption of the injured tissues.

* From the Pathological Department of Karolinska Institutet, Stockholm (Prof. F. Henschen).

1. Zenker: Dissertation, Leipzig, 1864.

2. Beneke, R.: Virchows Arch. f. path. Anat. **99**:71, 1885; Verhandl. d. deutsch. path. Gesellsch. **16**:403, 1913; Beitr. z. path. Anat. u. z. allg. Pathol. **22**:575, 1917; München, med. Wchnschr. **46**:1303, 1918.

Thoma³ and Weihl believed the degeneration to be due partly to mechanical causes and partly to disturbances in nutrition. Thoma, making observations on living frogs under the microscope, noted a waxy degeneration appearing in muscles of the tongue which he had irritated by means of a fine needle.

An excellent interpretation of waxy degeneration was published by an American author, Wells.⁴ By the experimental route, he came to the conclusion that the changes were brought about by lactic acid formed in the muscles. Bacteria and their toxins cannot by themselves give rise to degeneration, but they are the means of producing lactic acid in the muscle. Production and accumulation of large quantities of lactic acid may also be increased by a defective circulation through the injured muscles.

A number of authors have recently described waxy degeneration in various disease conditions. Postmortem specimens of muscles from new-born children were investigated by Beneke, who found waxy degeneration in all septic cases. Stemmler carried out systematic examinations of the muscles in twenty-five cases of infectious disease, and found waxy degeneration in all of them. Both Beneke and Stemmler found the greatest changes in the diaphragm, which is considered to be more disposed toward this degeneration than other muscles on account of its tension and continuous activity. According to these authors, the causative agent is probably some poison directly affecting the healthy fibrils.

Since the great influenza epidemic of 1918, a great number of authors have described muscular changes occurring in this disease. These changes have consisted chiefly of waxy degeneration and hemorrhages in the following muscles: rectus abdominis, the pectorals, the diaphragm and the cervical muscles (Oberndorfer,⁵ Schminke, Borst,⁶ Siegmund,⁷ Marchand,⁸ Schmorl, Askanazy,⁹ Hedinger, Glaus and Fritzsche,¹⁰ Wegelin¹¹). In regard to the localization, Kuczynski and Wolff¹² point out that the additional demand made on the respiratory

3. Thoma: Virchows Arch. f. path. Anat. **186**:64, 1906; **195**:93, 1909; **200**:22, 1910.

4. Wells: J. Exper. Med. **11**:1, 1909; Beitr. z. path. Anat. u. z. allg. Pathol. **21**:945, 1912.

5. Oberndorfer: München. med. Wchnschr. **30**:811, 1918.

6. Borst: München. med. Wchnschr. **48**:1342, 1918.

7. Siegmund: Med. Klin. **4**:95, 1918.

8. Marchand: München. med. Wchnschr. **5**:117, 1919.

9. Askanazy: Deutsches Arch. f. klin. Med. **61**:118, 1898; Cor.-Bl. f. schweiz. Aerzte **15**:465, 1919.

10. Glaus and Fritzsche: Cor.-Bl. f. schweiz. Aerzte **34**:1121, 1918.

11. Wegelin: Cor.-Bl. f. schweiz. Aerzte **49**:1478, 1919.

12. Kuczynski and Wolff: Ergebn. d. path. Anat. u. Pathol. **19**:947, 1921.

muscles in these cases would play some part. These authors give as causative factors of the degeneration: "the external and internal asphyxiation," the pathologic disintegration of the proteins brought about by influenzal toxins and perhaps by the increased function of the abdominal and cervical muscles and of the diaphragm occasioned by the pneumonic condition. Askanazy considered Zenker's degeneration to be due to ecchymosis, and Siegmund and Koopmann,¹³ to thrombosis in small muscular vessels. Utsumi described the changes in the skeletal muscles and peripheral nerves that occur during influenza. He found also waxy degeneration in almost every case; it was most pronounced in the abdominal recti, in the intercostal muscles and in the diaphragm. In 1923, this author made a systematic study of the skeletal muscles in different diseases. He found waxy degeneration in a great many muscles in infectious diseases. According to Utsumi, the muscles in children usually show only slight changes and waxy degeneration is present occasionally. In some cases he found a certain parallelism between muscular and nervous changes, but as this was infrequent, he believes that some damaging agent affects the muscular system itself.

I have examined the skeletal muscles in forty-seven patients that died from various diseases, of whom thirty-one were adults and sixteen children. In none of these cases was the degeneration absent altogether; in only one or two cases did it fail to appear in some muscles. The greatest changes were usually found in the diaphragm. Contrary to Utsumi,¹⁴ I have not been able to find any obvious difference in the changes that occur during different ages. As the relation between parenchymatous degeneration and rise of temperature has recently been under discussion, and as the occurrence of morphologic changes resulting from variations in the body temperature has become the subject of renewed observation (Fahraeus), I made an investigation to determine whether waxy degeneration has any connection with febrile conditions before death. As will be seen from the table, however, apparently the temperature of the patient does not have any influence on the occurrence or intensity of the degeneration.

FATTY DEGENERATION

Saikowski,¹⁵ in 1865, found fat in the diaphragm and heart in cases of arsenical poisoning of short duration. Gies made similar observations in cases of the same condition but of longer duration. Rindfleisch and Ziegler considered fat in skeletal muscles to be pathologic. Erhardt,¹⁶ in

13. Koopmann: *Virchows Arch. f. path. Anat.* **228**:319, 1920.

14. Utsumi: *Verhandl. d. jap. path. Gesellsch.* **10**:169, 1920; *Tr. Japanese Path. Soc.* **11**:42, 1921; **12**:16, 1922; **13**:96, 1923.

15. Saikowski: *Virchows Arch. f. path. Anat.* **34**:73, 1865.

16. Erhardt: *Beitr. z. path. Anat. u. z. allg. Pathol.* **23**:940, 1896.

1896, examined muscles from a rabbit with trichinosis and found fatty as well as waxy and hydropic degeneration. On examination of muscles from patients with trichinosis, however, definite fatty changes were not found.

Askanazy and others describe fatty degeneration of the skeletal muscles in exophthalmic goiter, and similar changes have been described by Langhans in cretinism and by Arnold in acromegaly. Askanazy considered the changes toxic in nature; according to Lemke, they were due to toxin produced by the thyroid.

Wahlbaum¹⁷ found fatty changes in the skeletal muscles of children with rickets, he does not infer, however, that there is any relation between the two conditions, as he found similar changes to the same extent in children not affected with rickets. The latter, however, were not normal children, as they all had died from some infectious disease. Wahlbaum found, as Hansemann did previously, fat in the levator palpebrae muscles in about 90 per cent of all cases examined by him and fatty changes in the skeletal muscles in about 40 per cent. The fatty degeneration was supposed, to some extent, to be dependent on the amount of work carried out by the individual muscles. This, however, is not true of the myocardium and diaphragm. In the latter organ, Wahlbaum found fat in only five of fifteen cases, and in only small quantities.

The occurrence of fatty changes in the muscular fibers after division of the nerve of the muscle has been described by several authors, such as Ricker and Ellenbach.¹⁸ Disturbances in the circulation are given as the probable explanation.

Zipkin¹⁹ and Langhans²⁰ found fatty changes in all the muscles after division of the cervical part of the spinal cord. The former author also found fat in skeletal muscles in a case of pneumonia and empyema.

Hester²¹ found fat in the skeletal muscles in tuberculosis, abscesses, colitis, enteritis, sarcoma, pneumonia, etc.; he points out that the fat drops are to be seen particularly about interstitial fatty tissue. From his experiments, he concluded that fat was split up by the normal tissue fluid and that the normal muscle fibers, when surrounded by an abundance of such fat, were capable of accumulating more fat.

According to Albrecht, fatty degeneration is due to an excess of those liposomes that occur particularly in the cytoplasm of muscles.

Fischer,²² in 1903, described a case of diabetes in which pronounced

17. Wahlbaum: Virchows Arch. f. path. Anat. **158**:170, 1899.

18. Ricker and Ellenbach: Virchows Arch. f. path. Anat. **158**:199, 1899.

19. Zipkin: Virchows Arch. f. path. Anat. **185**:478, 1906.

20. Langhans: Virchows Arch. f. path. Anat. **149**:155, 1897.

21. Hester: Virchows Arch. f. path. Anat. **164**:293, 1901.

22. Fischer: Virchows Arch. f. path. Anat. **172**:30, 1903.

lipemias occurred. Fat was found also, among other places, in the myocardium and in the skeletal muscles.

Keinarth,²³ in examining normal muscles for fat, found fat drops in muscles from various animal fetuses and full-grown animals. In man, Keinarth is said to have found fat in muscles from two executed persons as well as from two patients suffering from typhoid fever and meningitis, respectively. The fat was always between the fibrils and in a granular arrangement around the nuclei. In the two executed persons, the fat was within the muscular fibers and seen only with an immersion lens, being particularly noticeable at the polar ends of the nuclei. It may be questioned whether these really were fatty granules; judging from the description, it would seem more likely that it was brown pigment.

Ishida²⁴ gives an account of the occurrence of iron, microscopically demonstrable, as well as ferrous pigment in cross-striated muscles. The pigment, shown to originate from muscle hemoglobin, was observed in atrophic muscles. In nearly all the cases examined, there was in addition more or less fatty degeneration. The muscles were taken from persons who had died from a great variety of diseases. The muscles that had undergone most marked fatty changes, however, seemed to have been those from persons with infectious diseases, although great changes were noticed also in pernicious anemia, cancer, and other conditions. The diaphragm was generally the seat of the greatest changes. Waxy degeneration has also been observed in a good many such cases.

In 1918, Lipskra and Mlodowskra²⁵ gave an account of the relation between the glycogen and fatty contents in the muscles in the following words: "Das Mengenverhältnis der beiden im Skelettmuskulatur vorkommenden Stoffe ist im gesunden Körper wohl fast ausschliesslich von der Art der Ernährung abhängig, in dem Fettnahrung besonders zur Fettspeicherung, vorwiegende Kohlehydratnahrung hingegen besonders zur Glykogenspeicherung führt. Immerhin kann, bei letzterer Ernährungsart, wie Befunde bei Ratten und Kaninchen lehren, durch Fettsynthese auch eine ganz erhebliche Fettablagerung im Muskel zustandekommen." (The proportion of these two substances which are formed in skeletal musculature depends in a healthy organism almost entirely on the nature of the diet. A diet composed chiefly of fats causes especially an accumulation of fat; a diet composed chiefly of carbohydrates causes especially a deposition of glycogen. A carbohydrate diet may also result in a considerable deposition of fat in the muscles, depending on fat synthesis, which has been shown in experiments on rats and rabbits.)

23. Keinarth: Dissertation, Freiburg, 1904.

24. Ishida: Virchows Arch. f. path. Anat. **210**:67, 1912.

25. Lipskra-Mlodowskra: Beitr. z. path. Anat. u. z. allg. Pathol. **64**:18, 1917.

Utsumi found fatty degeneration of the diaphragm and of the gastrocnemius muscle in influenza. He says that such changes occur in skeletal muscles in other infectious diseases, and considers them to be due to disturbances of the metabolism in the muscle fiber itself.

Kolodny has based his work, "Ueber die Verfettung der willkürlichen Muskulatur" (1922), on the examination of about 200 cases of a great many different diseases. Fatty degeneration was found in the diaphragm in 55.7 per cent, in the tongue in 28.5 per cent, in the psoas in 17 per cent and in the perineal muscles in 1 per cent. In agreement with Lubarsch, Kolodny is of the opinion that absence of fat in the muscles is to be considered normal. In support of this assumption, he states that in fatty degeneration of muscles he has nearly always found similar changes in the myocardium and liver, and further, that such changes are almost never found in cases of sudden death (accidents, etc.). According to the same author, fatty degeneration is more common in the more active muscles, occurs in increasing quantities with age and is most frequent in chronic disease with serious circulatory disturbances. In the latter cases, he found fatty degeneration in muscles in 86.6 per cent and in diseases of the lungs and bronchi in 62 per cent. No relation seemed to exist between fatty degeneration and the general state of nutrition, and it was often accompanied by brown atrophy and waxy degeneration.

Fatty degeneration is mentioned as occurring in acute yellow atrophy of the liver by a great many authors, such as, Wagner,²⁶ Schultzen, Riess, Paulitzski, Huber, Herxheimer.²⁷

Case 23 in the table was that of a man, aged 36, who had had progressive muscular atrophy since the age of 17. He died from vitium cordis. At autopsy, changes in the nervous system were not found. Of the muscles, the following were found to be atrophic, all bilaterally: pectoralis major and minor, subscapularis, rhomboids, latissimus dorsi, biceps brachii, coracobrachialis, triceps, brachioradialis and gluteus maximus.

Briefly, the following are the changes observed in this disease: In addition to atrophic changes, fatty and waxy degeneration has frequently been observed. In most cases, however, the patients have succumbed from some intercurrent infectious disease. Preisz, Pappenheimer,²⁸ Pick, von Werdt,²⁹ Erb, Steinert and Versé found waxy degeneration but no fatty degeneration, which was described also by Münzer and Meyer. In my case there was moderate waxy degeneration in most muscles and fairly marked fatty degeneration.

26. Wagner: Deutsches Arch. f. klin. Med. **34**:520, 1884.

27. Herxheimer: Ergebn. d. path. Anat. u. Pathol. **8**:625, 1902; Beitr. z. path. Anat. u. z. allg. Pathol. **72**:56, 1923.

28. Pappenheimer: Beitr. z. path. Anat. u. z. allg. Pathol. **44**:430, 1908.

29. Von Werdt: Frankfurt. Ztschr. f. Path. **2**:577, 1908-1909.

In forty patients examined, fatty changes were not found in eight, of whom three had had tuberculosis, three pneumonia, one sepsis and one marasmus. In all the remaining cases the muscles had undergone more or less fatty degeneration, the individual muscle fibers generally being filled with fat granules of fairly small size. One or several apparently unchanged fibers, or fibers in which there are only waxy changes, are usually found between those that have undergone fatty degeneration. Sometimes the fatty granules may be seen within the flasklike swellings of the waxy fibers. Among the aforementioned muscles taking the stain for fat, seventeen were from persons over 50 years of age, of which sixteen showed fatty degeneration. Nine specimens were from persons between 50 and 20 years of age, of which seven had fatty degeneration. In a boy, aged 15, there were fatty changes in two muscles of three examined, and a child, 4 years old, had only slight fatty degeneration in the diaphragm. There were fourteen infants, and fatty changes were present in nine; a small amount of fat was found in four cases and in only one group of muscles.

In twenty-five of thirty-three instances, fat was found in the skeletal muscles, myocardium, liver or kidney.

No particular muscle seemed to be preferred, except perhaps the diaphragm; the intercostal muscles were often affected, but the pectoral and the sternocleidomastoid rarely. In all except two cases, waxy changes existed together with the fatty degeneration; this type of degeneration alone was present in two muscles.

There does not seem to be any connection between the fatty degeneration and the general state of nutrition. Further, this degeneration seems to occur more frequently in older persons; it occurs in 88.4 per cent of adults and in 62.5 per cent of children and infants. As examples of a marked degree of fatty degeneration may be mentioned cases 28, 16, 34 and 46, and as examples of cases in which fatty changes were not found, cases 8, 24, 25, 36, 38, 41.

DISTRIBUTION OF FATTY DEGENERATION IN CASES EXAMINED BY AUTHOR

In the cases in which I had an opportunity to make an examination, fatty changes were found in various diseases as follows:

Tumors: Two cases, 1 and 6, both showed a fairly general marked fatty degeneration. Fat was not found in one of the muscles examined in both cases, and fatty degeneration was present in the internal organs.

Tumor and Pneumonia: Four cases (4, 14, 15, 31); an almost general, fairly marked fatty degeneration of the muscles was present in addition to fatty changes in the internal organs.

Tumor and Sepsis: One case (17); a fairly slight fatty degeneration of the muscles was present.

Pneumonia: Six cases in adults (3, 5, 18, 25, 26, 27) and seven in children and infants (32, 35, 36, 39, 40, 13, 44); in the former group, two showed a general, fairly marked fatty degeneration. In one of these, the liver also showed similar changes. Three cases were entirely free from fat. In one case there was fatty degeneration in two muscles and none in two others. Case 32, that of a boy, aged 4, was almost free from fatty changes. Of the group of infants, one case showed fairly marked degeneration and one a moderate degeneration; in the remaining cases there were almost no changes of this kind. Independently of this, there were occasionally fatty changes in internal organs.

Sepsis: Seven adults (8, 9, 13, 16, 21, 22, 28) and three children and infants (33, 43, 45); in the former group, there was fairly marked to marked fatty degeneration. In all of the cases there were fatty changes in some internal organ. A 2 year old boy showed fairly marked fatty degeneration and two infants were almost entirely free from fat.

Sepsis and Pneumonia: Five cases from adults (2, 10, 11, 29, 30) and one infant (46); in the former group, the fatty changes were somewhat unevenly distributed in the different muscles, but in no case were they absent altogether. There were no demonstrable concurrent changes in the internal organs. Case 46, that of a boy, aged one-half month, showed exceedingly marked fatty degeneration in all muscles examined.

Miliary Tuberculosis: Two infants (34, 38); one showed considerable fatty changes; the other, none.

Tuberculous Enteritis: One case (19); there was fatty degeneration of the diaphragm only.

Atrophic Liver and Pneumonia: One case (7); there was fairly marked fatty degeneration; the diaphragm, however, was free from degenerative changes.

Diabetes Mellitus: One case (24); fatty changes did not occur in the muscles, but there were changes in the myocardium and liver.

Muscular Atrophy: One case (23); there were fairly marked changes in most muscles.

Marasmus: One case (41) in an infant; fatty changes were not seen.

Debility and Pneumonia: One case (47) in an infant; six muscles examined, one showed moderate fatty degeneration, as did the liver.

Apparently, definite relation does not exist between fatty degeneration and any particular disease. In tumors, however, fatty degeneration apparently occurs frequently; in the septic cases of the series, it was constant.

PIGMENT

Brown pigment around the poles of the nuclei occurs only in advanced age, being fairly common after the age of 50. In the case of a sailor, aged 43, however, who died from amebic dysentery, pigment

was present in fairly great quantities. This man had always been engaged in hard work and showed considerable wasting before death. Concurrent atrophy of muscular fibers could not be demonstrated; on the contrary, these were of greater width than normal fibers. The term "brown atrophy" would therefore seem inappropriate in such cases.

The technic used was the usual one, except that, to avoid shrinkage, etc., the preparations were fixed on paper prior to the treatment with formaldehyde; in some cases they were fixed on cardboard by means of fine wires. The frozen sections were stained with scharlach R and Weigert's hematoxylin; the paraffin sections, with van Gieson or hematoxylin-eosin.

For the sake of comparison, the myocardium, liver and kidney were examined also in some cases. It was found that fatty degeneration in muscles is generally accompanied by similar changes in internal organs. As instances in which only muscular degeneration occurred may be mentioned cases 10, 11, 29, 30 and 35. The following cases showed fatty changes of the internal organs, but not any in the muscles: 24, 25, 39, 19.

NOTE.—In the September, 1926, issue of the ARCHIVES Forbus gave an account of degeneration and regeneration of the abdominal recti in pneumonia. Almost all of the pictures of degeneration described by him accord well with the observations I have made, not only in pneumonia but also in many other diseases.

SUMMARY

The skeletal muscles were examined in forty-seven cases of different diseases at various ages with regard to waxy and fatty degeneration and to brown pigmentation; in all, they proved to be more frequent and general than hitherto known.

More or less waxy degeneration was found in all cases, most marked in the diaphragm, but without distinct difference for various age groups and without any relation to the degree of fever.

More or less pronounced fatty degeneration was found in thirty-two of forty cases. The diaphragm was possibly a little more often the seat of the changes than the other muscles. In almost every case, waxy degeneration was present also. There was not any obvious relation to the general individual state of nutrition. In eighty-eight adults, 4 per cent, fatty degeneration was observed. Any distinct relation between special diseases and muscular fatty degeneration was not established, but in cases of tumor the degeneration seems to be frequent and in cases of sepsis in adults it is constant. Often there is fatty degeneration of the heart, liver and kidney at the same time.

Brown pigmentation was found only in muscles of persons over 50 years of age.

TABLE 1.—Summary of the Cases*

Case	Age	Sex	State of Nutrition	Temp.		Diagnosis	Mus- cles Exam- ined	Waxy De- gen- era- tion	Brown Pig- men- tation	Fatty De- gen- era- tion	Fat in		
				De- grees, C.	Dura- tion, Days						Myo- car- dium	Liver	Kid- ney
1	83	F	Wasted	Cardio-arteriosclerosis, carcinoma of rectum, general anemia	D I-C S-M	++ ++ +	++ — —	++ ++ —	++	+++	++
2	82	M	Very wasted	38-39	3	Purulent prostatitis, cystopyelitis, bron- chopneumonia	D I-C P S-M R	++ + ++ ++ ++	++ — + ++ +
3	79	M	Ordinary	40 (about)	5	Arterioneurophrosclerosis, confluent pneumonia, cerebral hemorrhage	D I-C P S-M R	++ ++ ++ ++ +	— — — — —	++ ++ +++ ++ ++	..	+	..
4	77	M	Wasted	39 (about)	7	Cardio-arteriosclerosis, hypopharyngeal car- cinoma, acute double pneumonia	D I-C S-M R	++ ++ + +	++ ++ + +	++ ++ ++ +	—	+	+
5	76	F	Wasted	39 (about)	4	Encephalomalacia, bronchopneumonia	D I-C S-M R	++ ++ ++ +	— — + —	++ ++ ++ +
6	72	F	Stout	0	0	Cardio-arteriosclerosis, carcinoma of pancreas	D I-C P S-M R	++ ++ + ++ —	— — — — —	++ ++ + ++ —	+
7	72	F	Ordinary	39	4	Cardio-arteriosclerosis, subacute atrophy of liver, broncho- pneumonia	D I-C P S-M R	++ + + + —	— — — — —	+++ +++ +++ +++ —	—	++	..
8	70	M	Wasted	38 (about)	3	Cardio-arterioneurophro- sclerosis, purulent parotitis, encephalo- malacia	D I-C P S-M	++ 0 ++ ++	0 0 0 0	++ + ++ +	..	+	..
9	69	F	Ordinary	40 (about)	2	Cardio-arterioneurophro- sclerosis, chronic pur- ulent cystitis, right purulent parotitis, tabes dorsalis	D I-C S-M R	++ + ++ +	0 0 0 0	++ ++ ++ +	++	+	+
10	68	M	Wasted	39 (about)	7	Cardio-arteriosclerosis, catarrhal pneumonia, purulent pros- tatitis	D I-C P S-M R	+++ ++ ++ ++ 0	++ ++ ++ ++ 0	(+) 0 ++ ++ 0	0	0	0
11	66	M	Ordinary	39 (about)	8	Brachial phlegmonous erysipelas, broncho- pneumonia	D I-C P S-M R	+++ ++ + 0 +	0 0 0 0 0	++ ++ + 0 0	0	0	0
12	65	M	Tuberculous pericar- ditis, double pneu- monia, acute pur- ulent appendicitis	D I-C P S-M S-hy R	++ ++ 0 ++ ++ +	0 0 0 0 0 0
13	65	F	Stout	38 (about)	30	Arteriosclerosis, facial erysipelas	D I-C P S-M R	++ + ++ ++ +	0 ++ ++ 0 +	++ + ++ 0 0
14	65	F	Very wasted(abou)	39 (about)	2	Cardio-arteriosclerosis, right catarrhal pneu- monia, bilateral, pur- ulent pyelonephritis, ulcerative carci- noma of uterus	D I-C S-M R	++ ++ ++ ++	++ ++ ++ ++	++ ++ ++ 0	+	+	0

* D indicates diaphragm; I-C, intercostals; P, pectoralis major; S-M, sternocleidomastoid; R, rectus abdominis; I-p, iliopsoas; S-hy, sternohyoid. The degree of degeneration is shown thus: (+), slight; +, moderate; ++, fairly marked; +++, very marked; absence of changes, 0 or —.

TABLE 1.—Summary of the Cases—Continued

Case	Age	Sex	State of Nutrition	Temp.		Diagnosis	Mus- cles Exam- ined	Waxy De- gen- era- tion	Brown Pig- ment	Fatty De- gen- era- tion	Fat in		
				De- grees, C.	Dura- tion, Days						Myo- cardium	Liver	Kid- ney
15	64	M	Very wasted	38 (about)	6	Cardio-arterionephro- sclerosis, catarrhal pneumonia, bilateral hypernephroma	D I-C S-M R	++ ++ ++ ++	0 0 0 +	+++ 0 + +	..	+	..
16	64	F	Wasted	38 (about)	30	Gangrene of maxillary antrum, diphtheria, ulcerative colitis, fibropurulent peritonitis	D I-C S-M R	+ + — +	0 0 0 0	+++ + 0 (+)	..	+	0
17	63	M	Wasted	39 (about)	14	Cardio-arteriosclerosis, left renal tumor, purulent pyle- phlebitis	D S-M R	++ + (+)	+	0 + (+)	+	0	..
18	61	F	Wasted	38 (about)	6	Acute and chronic pul- monary tuberculosis, right purulent pneumonia	D I-C P S-M R	+ + + + +	.. + + + 0	.. 0 0 0 0
19	55	M	Wasted	0	0	Pulmonary tuberculo- sis, tuberculous ulcers of intestines	D I-C S-M	+ ++ ++	0 + +	+	..	+	..
20	48	M	Wasted	Acute and chronic pul- monary tuberculosis and tuberculous ulcers of intestines	D I-C S-M R	+ ++ ++ ++	0 0 0 0
21	43	M	Wasted	39 (about)	30	Amebic dysentery, right pulmonary abscess, liver abscess	D I-C R	+ ++ +	+	0 + +	+	+	0
22	39	M	Wasted	40 (about)	7	Multiple septic infarcts of lungs, tuberculous bronchial glands	D I-C S-M R	++ + + +	0 0 0 0	0 ++ ++ +
23	36	M	Wasted	0	0	Progressive muscular dystrophy	S-M P I-C D Serrat. Biceps Deltoid Vast. fem. Sartor. Gastrocn.	+ 0 + + — + + + ++ (+) ..	0 0 0 0 0 0 0 0 0 0 +	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ +
24	36	M	Diabetes mellitus, at- rophy of pancreas, tuberculosis of cervical glands	S-M I-C R	0 + 0	0 0 0	0 0 0	+	+++	0
25	35	F	Wasted	40 (about)	7	Right pneumonia and right fibrinous and hemorrhagic pleurisy	D I-C P S-M R	++ 0 0 0 +	0 0 0 0 0	0 0 0 0 0	0	+	0
26	34	F	Ordinary	39.5 (about)	7	Right pneumonic influenza	D I-C P S-M R	++ ++ + 0 +	0 0 0 0 0	0 + + 0 0	0	+	0
27	30	F	Wasted	39 (about)	7	Catarrhal pneumonia, acute coloproctitis	D I-C S-M R	+ + + +	0 0 0 0
28	28	M	Very wasted	40 (about)	4	Sepsis, endocarditis, right tonsillar abscess	D I-C P S-M R	++ + + + +	0 0 0 0 0	++ +++ ++ +++ +++	0	+	0
29	23	F	Ordinary	38 (about)	90	Chronic fibrous and acute ulcerative endo- carditis, bronchopneu- monia, embolic focal nephritis	D I-C P S-M R	++ + (+) + 0	0 0 0 0 0	+	0	0	0

TABLE 1.—Summary of the Cases—Continued

Case	Age	Sex	State of Nutrition	Temp.		Diagnosis	Mus. Examined	Waxy De-cles-eration	Brown Pig-ment	Fatty De-gen-eration	Fat in		
				De-grees, C.	Dura-tion, Days						Myo-cardium	Liver	Kid-ney
30	21	M	Wasted	39 (about)	3	Septic periproctitis, acute purulent prostatitis, acute fibrinopurulent peritonitis, gangrenous bronchopneumonia	D I-C S-M R	+++ + — 0	0 0 0 0	++ 0 0 0	0 0 0 0	0 0 0 0	0
31	15	M	Wasted	Cerebral tumor, morbilli, bronchopneumonia	D I-C R	++ ++ 0	0 0 0	(+) ++ 0
32	4	M	Wasted	39.5 (about)	7	Acute fibrinous pleuritis, bronchopneumonia	D I-C S-M R	(+) ++ (+) +	0 0 0 0	(+) 0 0 0	0	+	0
33	2	M	Ordinary	39 (about)	5	Subacute phlegmon in pectoralis major (right) and phlegmonous mediastinitis	D I-C P R	+ ++ ++ +	0 0 0 0	0 ++ ++ +++	..	+++	..
34	6.5 mo.	M	Ordinary	39 (about)	7	Tuberculosis of mediastinal glands, general tuberculosis, tuberculous meningitis	D I-C P S-M R I-P	+ + +++ + + (+)	0 0 0 0 0 0	+++ +++ ++ + + —	++	+++	+++
35	6	F	Ordinary	Rickets, pneumonia	D I-C P R I-P	++ ++ ++ +++ +	0 0 0 0 0	+ (+) +++ — (+)	0	0	0
36	5.5	M	Ordinary	40 (about)	15	Pneumonia, fibrinous pericarditis and pleuritis (left)	D I-C P S-M R I-P	+ + 0 0 0 +	0 0 0 0 0 0	0 0 0 0 0 0	0	0	0
37	4.5	F	Wasted	40 (about)	3	Debility, pharyngitis, left-sided pleuritis	D P S-H R I-P	++ + (+) ++ +	0 0 0 0 0	+	..
38	4	M	Wasted	38 (about)	14	Miliary tuberculosis, tuberculous meningitis	D I-C I-P	++ + +	0 0 0	0 0 0
39	3	F	Wasted	Purulent pneumonia, fibrinopurulent pleuritis	D I-C P S-M R	++ ++ +++ ++ +	0 0 0 0 0	0 0 0 0 0	+	+++	+
40	3	M	Ordinary	40 (about)	3	Bronchitis and diffuse bronchiolitis	D I-C P S-M R	++ + (+) ++ +	0 0 0 0 0	+	0	++	+
41	2	M	Very wasted	0	0	Pyloric spasm, marasmus, catarrhal pharyngotracheobronchitis	D I-C P S-M R	++ + ++ ++ (+)	0 0 0 0 0	0 0 0 0 0	0
42	1.5	M	Ordinary	39 (about)	7	Purulent pharyngotracheobronchitis, bronchopneumonia	D S-M R I-P	+++ + ++ (+)	0 0 0 0	0 0 0 0	0	+	+
43	1	M	Wasted	38.5 (about)	7	Necrosis umbilici, acute fibrinopurulent peritonitis	D I-C S-M R	++ ++ (+) ++	0 0 0 0	0 0 0 (+)	0	+	+
44	1	F	Satisfactory	Subf.	14	Mucopurulent bronchitis	D I-C P I-P	++ ++ + +	0 0 0 0	0 + + +

TABLE 1.—Summary of the Cases—Continued

Case	Age	Sex	State of Nutrition	Temp.		Diagnosis	Mus- cles Exam- ined	Waxy		Fatty	Fat in		
				De- grees, C.	Dura- tion, Days			De- gen- era- tion	Brown Pig- ment		Myo- car- dium	Liver	Kid- ney
45	½	M	Ordinary (about)	38	14	Sepsis umbilici, retro-peritoneal abscess, purulent ileofemoral arthritis	D	++	0	0	0	0	0
							P	(+)	0	0			
							S-M	0	0	0			
							R	0	0	0			
							I-p	+	0	0			
46	¼	M	Satisfactory (about)	38	2	Infected umbilicus, purulent pharyngo-tracheobronchitis, bilateral pneumonia	D	+	0	+++
							I-C	+	0	+++			
							P	+	0	+++			
							S-M	+	0	+++			
							R	+	0	+++			
47	½	F	Very wasted	Congenital debility, hemorrhagic pneumonia	I-p	+	0	+++			
							D	(+)	0	0	0	+	0
							I-C	+	0	0			
							P	+	0	0			
							S-M	(+)	0	+			
							R	(+)	0	0			
							I-p	(+)	0	0			

DIFFUSE TUMORS OF THE LEPTOMENINGES

TWO CASES IN WHICH THE PROCESS WAS REVEALED
ONLY BY THE MICROSCOPE *

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AND

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In the literature dealing with brain tumors, one finds numerous reports of cases in which there has been a more or less widespread dissemination of the growth through the leptomeninges. Ordinarily, the primary lesion which has served to inoculate the spaces is readily determined. Thus, the meninges in the vicinity of a glioma of the cortex may be thickly matted together by an obvious local invasion of tumor cells; in other cases, more or less distant and easily discernible implantations of tumor growth may be plastered over the cerebrum, cerebellum or spinal cord.

In certain rare cases, however, the primary lesion may be so minute, and the meningeal infiltration so thinly spread, as to be invisible to the naked eye. Under these circumstances the tumor may be disclosed only after a microscopic examination of the tissues. Without such an examination the first of the two cases herewith reported might have gone on the hospital records as one of "brain tumor suspect—probable cerebral arteriosclerosis," and the second as one of "pseudo-tumor cerebri," both being equivocal classifications intimating that a lesion clearly accounting for the clinical symptoms had not been found.

CASE 1.—A man past middle age with obscure meningeal symptoms. Cerebral arteriosclerosis the only gross postmortem observation. Histologic disclosure of unsuspected meningeal invasion by tumor.

Henry S., aged 63, of Swedish descent, a laster in a shoe factory, was admitted on Nov. 19, 1925, with the following history:

Clinical History.—In the latter part of September, 1925, he had been obliged to cease work because of dizziness, weakness and indefinitely localized pains in the joints, chest and abdomen. Four weeks later, on October 20, he was admitted to the Brockton Hospital in a semistuporous condition, with a temperature of 101.6 F., high systolic blood pressure and symptoms indicating some intracranial disorder. The urine contained a trace of albumin, pus cells and, on several occasions, many hyalin and granular casts. His condition was ascribed to vascular hypertension and chronic nephritis.

On five occasions between October 23 and November 15 a lumbar puncture had been performed. The fluid on each occasion was straw colored, with an exceptionally high initial pressure varying from 290 to 430 mm. The Wassermann reaction

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from both the spinal fluid and the blood proved negative. On November 9, there was an outcrop of thoracic herpes zoster. He grew progressively worse, became incontinent, incoherent and uncooperative, and was transferred to the Brigham Hospital for neurologic study.

Physical Examination.—On admission this revealed little more than had already been observed. The patient was somewhat emaciated and semicomatose, with a herpetic eruption over the right costal margin, a mild chronic nephritis and a blood pressure of 185 systolic and 100 diastolic. A stiff neck, a positive Kernig sign and considerable rigidity of the upper extremities were observed. The pupils were dilated, at times unequally so, and without reaction. The eyegrounds were negative except for marked arteriosclerosis. Roentgenograms of the skull were negative.

Lumbar puncture showed yellow, slightly turbid, opalescent fluid, alcohol +++; ammonium sulphate ++; red blood corpuscles 48,000; white corpuscles 35; polymorphonuclears 30 per cent; large mononuclears 50 per cent; small mononuclears, 25 per cent; no bacteria; Wassermann reaction negative; colloidal gold curve 2151051. The fluid was sterile. This observation, in the absence of a history of a recent faulty lumbar puncture, suggested that there might be an absorbing meningeal hemorrhage possibly from a ruptured mycotic aneurysm or subdural hematoma.

Discounting the blood tinged fluid, however, a differential diagnosis lay between cerebral arteriosclerosis, tumor, encephalitis and meningitis of unknown origin.

Because of the obscurity of the case and the absence of pressure signs operation was not undertaken. Without any essential modification in the symptoms the patient's condition went from bad to worse during the succeeding three weeks. He finally became cyanotic, breathed stertorously, and died on December 3.

Autopsy.—This was restricted to an examination of the head. The brain was removed by Dr. Davidoff after it had been fixed in situ with intracarotid injection of a 10 per cent solution of formalin. The cerebral surface showed a number of minute ecchymotic spots which were visible through the dura. There was not, however, evidence of any recent extravasation in the subarachnoid space. The convolutions were normal in appearance and the leptomeninges did not show any evidence of thickening or other abnormality. The vertebral and basilar arteries were sclerosed and tortuous.

A series of coronal sections disclosed nothing more than a few minute ecchymotic spots in the white substance of the cerebellum (fig. 1). These spots were supposed to have been agonal in occurrence and were thought in all probability to be associated with the evident arteriosclerosis.

Under the impression that the case was one of vascular disease rather than of tumor, and because of Dr. Bailey's absence, the brain was transferred from the neurosurgical laboratory to the pathologic department for further study.

Pathologic Report (Dr. Connor).—The brain, as received, had been cut in several coronal planes. There was no evidence of tumor or of other gross lesion on the surface, apart from a considerable sclerosis of the basilar vessels and a possible slight flattening of the convolutions. A few tiny hemorrhages were present in the dura at the base.

Further coronal sections were made without additional information, aside from a rather indefinite obscuration of the usual markings in the left cerebellum in the region below and lateral to the dentate nucleus in which a few punctate hemorrhages were present (fig. 1).

Routine blocks from the cortex, basal ganglia, pons and cerebellum were taken for microscopic examination. The selection was made somewhat haphazard as they were not expected to reveal any definite pathologic lesion. It consequently was a surprise to find a uniform, diffuse thickening of the basilar meninges produced by an infiltration which bore a superficial resemblance to a mononuclear cell reaction to infection (fig. 2). Moreover, in certain of the cerebellar gyri penetrating as far as the granular layer were numerous small perivascular accumulations of similar cells (fig. 3).

Further sections of the brain were then made, particular attention being paid to the cerebellum in which the process seemed to be most active. Though a definite primary focus of tumor could not be found, having the microscopic

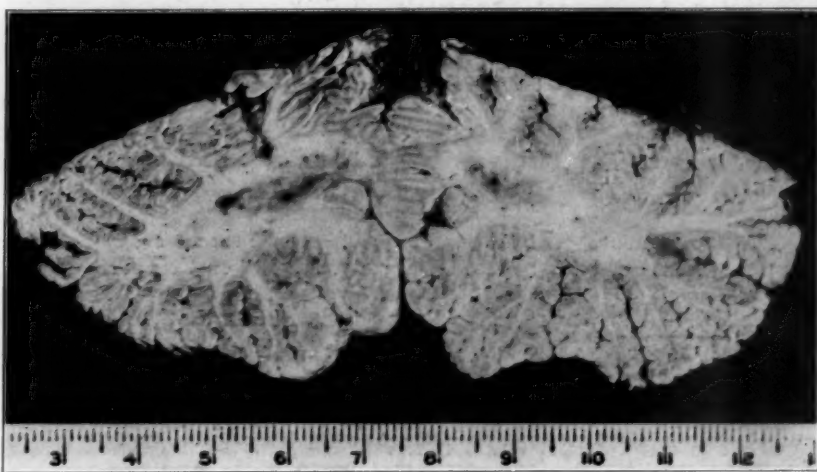


Fig. 1 (case 1).—Showing the only macroscopic observations, namely, small ecchymotic spots in the substance of the cerebellum; these proved to be contiguous to the primary tumor, but tumor cells were not found in or surrounding the hemorrhages.

sections as a guide, tiny masses of what might be tumor were made out with difficulty near the cerebellar borders with a hand lens. These scattered areas showed up only as fine mottlings and were scarcely to be distinguished from the normal brain. The meninges, however, could be seen to be thickened, chiefly in an area passing forward over the anterior part of the cerebellum along the superior cerebellar peduncles toward the pineal region.

Finally a large number of blocks were taken for microscopic examination. From first to last some 90 were cut, comprising all parts of the brain. Sections were stained with eosin-methylene blue, Giemsa's stain, phosphotungstic acid-hematoxylin, Hortege's fourth variant for neuroglia, Cajal's stain for nerve fibers and Perdrau's method for connective tissue. Among the larger blocks, one included half of the cerebellum and another the pons and peduncles at the level of the pineal body. Other sections were taken from along the brain stem, optic

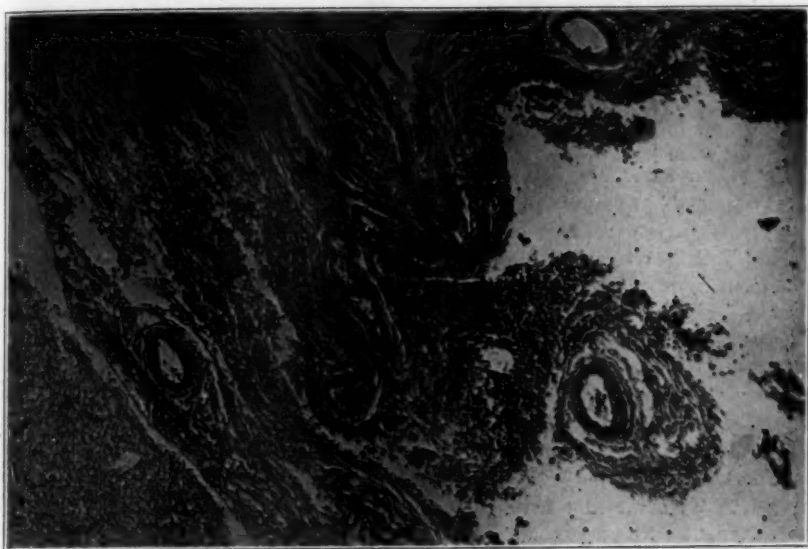


Fig. 2 (case 1).—The meningeal invasion bears a striking resemblance to an infectious meningitis. Eosin-methylene blue stain, $\times 80$.

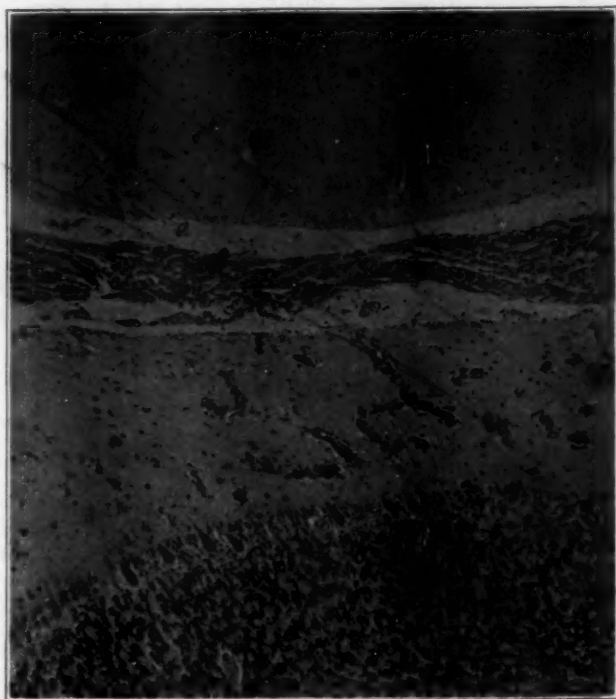


Fig. 3 (case 1).—Showing tumor invasion of meninges and also presence of tumor cells along the perivascular spaces of the lower cerebellar lobule.

chiasm, fifth nerve and ganglion, Pacchionian bodies, medulla and various places along the ventricular system. The description will be condensed as much as possible in the following paragraphs.

Histopathologic Report.—The chief perivascular accumulations of tumor cells in the brain substance were ultimately found to lie in the molecular layer of the anterior surface of the cerebellum, in the tonsil and in the gray matter below the dentate nucleus. The largest single accumulation (fig. 4) lay in the region of the foramen of Luschka and borders on the lateral diverticulum of the fourth ventricle on the left. The tissues around this area of chief involvement showed congestion and moderate degeneration. The adjacent



Fig. 4 (case 1).—The chiefly involved area of the cerebellum lies just beneath the dentate nucleus bordering on the lateral recess; this is considered to be the probable source of the adjacent meningeal infiltration. Hematoxylin and eosin stain. $\times 20$.

meninges were thickened with tumor, which was continuous with that in the brain substance around the vessels. The flocculus beneath this nodule, and the tonsils above and medial to it, showed strands and clumps of perivascular tumor cells.

The perivascular involvement was chiefly confined to the small vessels of the molecular layer of the cerebellum, and only here and there extended to the granular layer and still more rarely to the white substance. The tumor cells had practically not provoked any reaction on the part of the surrounding tissues. In certain areas the cells of Purkinje were separated and showed slight swelling indicative of impending degeneration. Hemorrhage had not occurred in the vicinity of the tumor; necrosis was not seen, and lymphocytes were not present.

From this, its presumed primary source, the tumor which had so extensively invaded the cerebrospinal spaces could be traced by serial sections. It had passed forward for a short distance in the fourth ventricle along the choroid plexus where the cells were intimately intermingled with the capillaries and stroma of the plexus. It extended along the meninges over the tonsils and uvula, and followed the perivascular spaces into these structures. Thence it spread forward in the spaces enveloping the superior cerebellar peduncles, the colliculi and pineal body. It was present in the choroid plexus of the suprapineal recess of the third ventricle, and at the anterior termination of the iter, though the latter apparently was not blocked. From this central region it spread down on both sides around the cerebral peduncles and the pons. It also passed upward and backward, around the superior border of the cerebellum, and spread out over this structure in thin sheets. It extended forward to involve the meninges around the optic tracts and chiasm, the trigeminal root and semilunar ganglion. It covered the cerebral cortex at the hippocampus and passed into a sulcus inferior to the left lateral ventricle. It had not spread for any distance over the cerebrum. Sections from the lateral and superior surfaces of the cerebral cortex, from the pachionian accumulations, from the optic nerves and from the medulla did not show any tumor.

The spread of tumor cells was therefore chiefly confined to the posterior and basilar meninges in the space between the temporal lobes from the clava of the medulla posteriorly to the pituitary gland anteriorly. In its manner of invasion and disposition the growth behaved much as would a basilar meningitis.

Comment.—The cytological details of the growth were primarily studied with the special stains already mentioned. As they occurred in the meninges (fig. 5), the tumor cells were of moderate size, round, oval or elongate, with abundant clear cytoplasm and a round or oval nucleus, rarely a nucleolus, the nuclear clumps being scattered and forming no particular pattern. On first appearance they suggested the cells of a meningioma (dural endothelioma), and the process was interpreted as a spreading endotheliomatosis which had originated in the perivascular expansions of the leptomeninges and had secondarily "infected" the cerebrospinal fluid pathway.

Though this, our first conception of the process, proved to be correct, we were temporarily led to distrust the diagnosis, since the cells were found to show an intercellular substance composed of fine and coarse wavy fibrillae (fig. 16). When stained with Hortega's fourth variant these fibrillae were at first mistaken for glial fibrillae, but further study has led us to believe that actual glia fibrillae (disclosed by neutral ethyl violet-orange G), are rare and can usually be seen to project into the meninges from the subpial fibrillary glia. The fibrillary material proper, as was shown by Perdrau's method, consisted almost exclusively of reticulum lying in intimate contact with the cells and collagenic fibers (fig. 6).

The case, therefore, as was our first impression, proved to be one of diffuse basilar endotheliomatosis due to an invasion of the cerebrospinal pathway, with tumor cells originating from the adventitial sheath of the blood vessels.

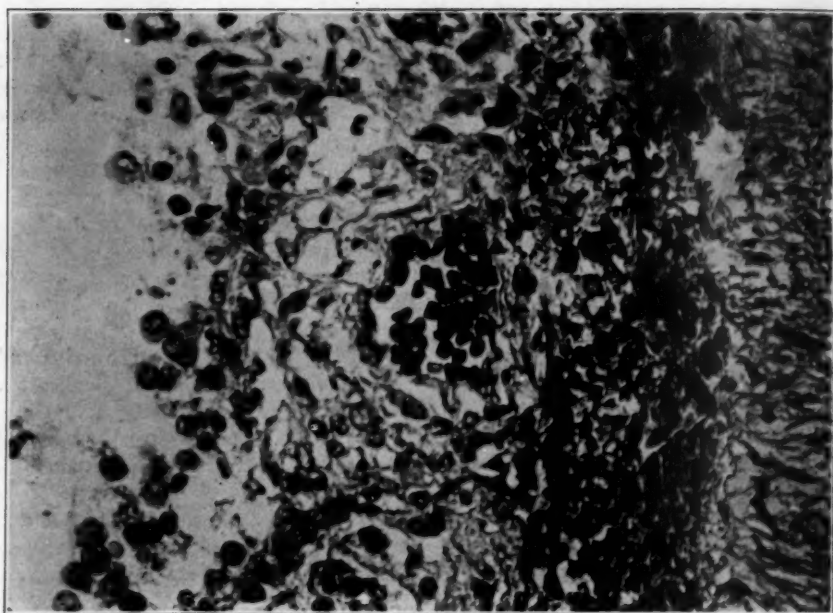


Fig. 5 (case 1).—Meningeal tumor from surface of brain stem, showing character of neoplastic cells; the free lying endothelial cells at the left, the brain to the right and a layer of collagenic tissue between should be noted. Neutral ethyl violet-orange G. $\times 400$.

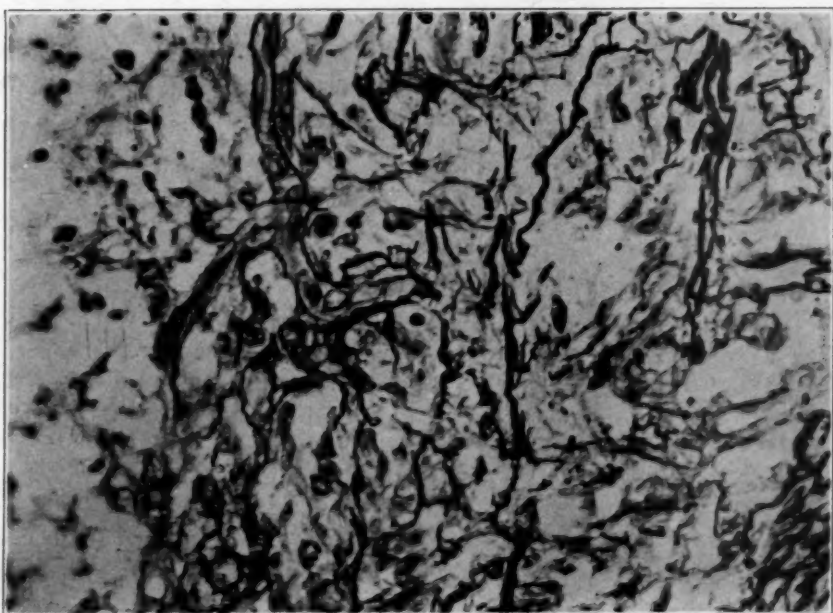


Fig. 6 (case 1).—Meningeal tumor showing reticulum in intimate contact with neoplastic cells. Perdrau's method. $\times 400$.

While this brain was being studied in the pathologic laboratory the combination of circumstances which led to its transfer from the neuro-surgical laboratory was repeated, namely, the transfer of another brain which in gross showed no evidence of tumor, though an operation had been performed for such. It also proved histologically to have a meningeal tumor invasion.

CASE 2.—A child with a definite cerebellar syndrome. Operation with negative results. Death four weeks later, with autopsy. Provisional diagnosis "pseudo-tumor cerebri;" histologic disclosure of meningeal gliomatosis.

Howard T., aged 11, an American schoolboy, was admitted, April 27, 1926, referred from the Children's Hospital by Dr. Bronson Crothers because of rapidly failing vision and with the diagnosis of cerebellar tumor.

Past History.—The patient was one of a large family and had never been particularly vigorous. He had had measles at the age of 6, three attacks of tonsillitis and an attack of otitis media; he had also had periodic attacks of vomiting since early infancy.

Present Illness.—This began in September, 1925, seven months before admission, when the child began to complain of pain in the lumbar region and coccyx. He continued to attend school in spite of oncoming headaches, vertigo and some unsteadiness. Finally, one day, he was sent home because of projectile vomiting while in class. The symptoms grew progressively worse and for the few weeks before admission he had been confined to bed with increasing headaches, vomiting, ataxia and failing vision. For a time he had diplopia, but this disappeared when vision failed completely in the right eye.

Physical Examination.—The boy was undeveloped and poorly nourished though mentally alert and cooperative. The head gave a cracked pot sound on percussion. The roentgen ray showed slight separation of the sutures; also deepened convolutional impressions with an enlarged sella suggesting hydrocephalus. There was a bilateral choked disk of about 3 diopters, with marked secondary atrophy. Vision was greatly reduced, with total amaurosis in the right eye. This eye tended to turn outward and upward with an obvious squint.

He was unable to walk without support because of staggering, and in the Romberg position he would fall backward. There was marked hypotonia and incoordination. Nystagmus was not present.

A median cerebellar, possibly fourth ventricle tumor, was suspected.

On May 1 a suboccipital exploration was performed; the ventricular fluid was small in amount and not under tension. On exposing the cerebellum a slight foraminal herniation was disclosed. To reduce the tonsils fully and get a good view of the fourth ventricle the laminae of the atlas had to be removed. The arachnoid apparently was not thickened. The calamus scriptorius and lower angle of the fourth ventricle were to all appearances normal.

The ventricular fluid proved to be normal, clear, colorless and without cells; globulin 0; colloidal gold curve, negative; total protein from 9 mg. to 100 cc.

There were no surgical complications; the wound healing was perfect. Nothing, however, was gained by the operation, not even the usual palliative benefits of a suboccipital decompression. Though postoperative bulging of the wound indicating tension was not observed, the headaches continued, the child became completely blind, vomiting persisted, and the neck tended to be retracted. Had there been fever, a tuberculous meningitis might have been suspected. Finally, on June 4, a month after the operation, a sudden convulsion ended the story.

Autopsy.—In this case a complete postmortem examination was held. This showed merely a slight early bronchopneumonia and a chronic otitis media on the right side. The brain was removed by Dr. Davidoff after the usual fixation in situ with 10 per cent formalin. The cerebrum showed no convolutional flattening. The superficial leptomeninges were smooth and glistening, and were regarded as being normal everywhere. The cerebellum, however, appeared to be distinctly large, and it was thought that sections would surely reveal a gross tumor.

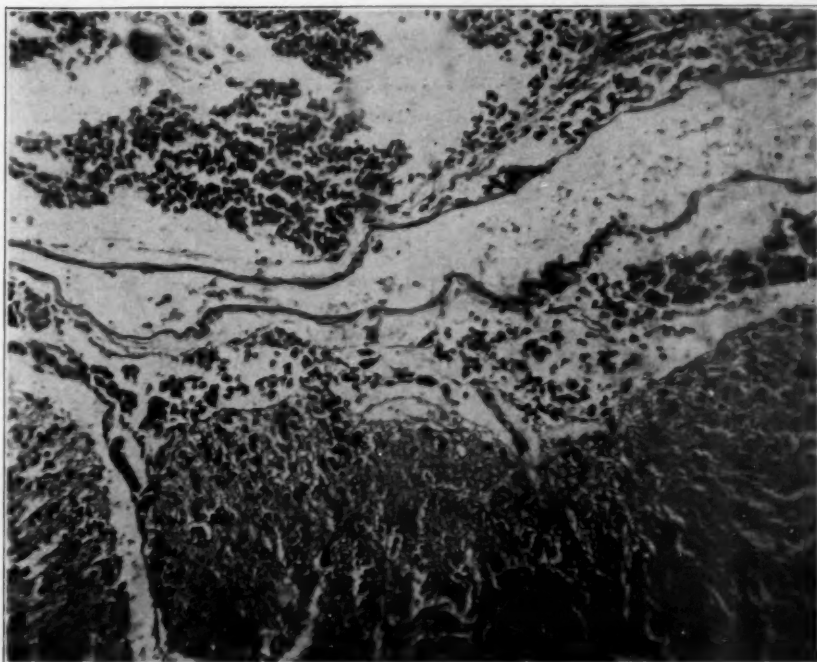


Fig. 7 (case 2).—Cerebellum and leptomeninges infiltrated with tumor cells. Eosin-methylene blue stain. $\times 80$.

Nevertheless, when the brain was sectioned no tumor was found. The ventricles were not dilated, and the general markings appeared normal in all respects. A series of thin coronal sections were taken, and the only apparent abnormalities were: (1) a possible grayish thickening of the leptomeninges in the region of the velum interpositum; and (2) a hemorrhagic spot partly obscuring the left dentate nucleus. Blocks of tissue were taken from these regions for study. If they had shown nothing beyond some thickening of the meninges the case would have gone on record as an example of "pseudo-tumor cerebelli."

Histopathologic Report (Dr. Connor).—The original blocks of tissue submitted for study showed, in the leptomeninges, thin sheets of cells which were

undoubtedly tumor (fig. 7). These cells were comparatively small, were round or oval, and contained round nuclei. The nuclear chromatin was somewhat scattered and formed no particular pattern, and nucleoli were rarely present. The cytoplasm was small in amount, stained blue in eosin-methylene blue preparations, and occasionally also with phosphotungstic acid-hematoxylin.

In the cerebellum itself the tumor was infiltrating along the perivascular spaces, and appeared as small groups of cells in the molecular layer, and, in places, even in the granular layer. Cells were not present in the white matter, and tumor cells were nowhere abundant.

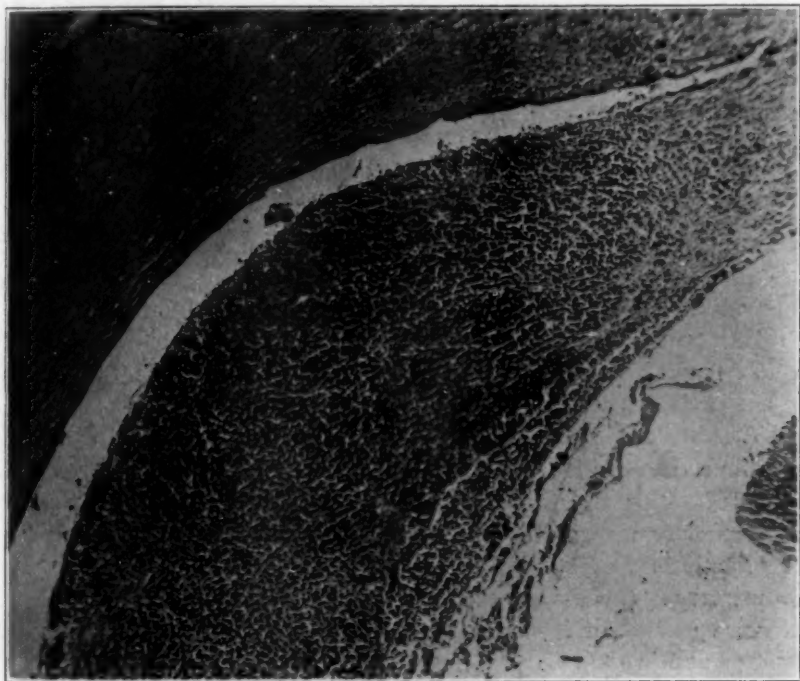


Fig. 8 (case 2).—A small mass of tumor projecting into the fourth ventricle; the lesion is in almost the same situation as the primary area of tumor involvement in case 1 (fig. 4). Phosphotungstic acid-hematoxylin stain. $\times 20$.

In view of the foregoing observation some thirty blocks of tissue were subsequently taken for study. These included the following regions: the cerebellum, including various parts along the fourth ventricle; iter and peduncles; roof and floor of lateral ventricles; medulla; cortex of occipital lobes; thalamus and third ventricle; optic tract; optic chiasm; olfactory bulb; pituitary body.

Tumor was present in the greatest abundance in the left cerebellum near the lateral diverticulum of the fourth ventricle, where a small microscopic nodule, doubtless the primary tumor (fig. 8), projected into the ventricle for a short distance. Beneath it the flocculus, and above it the tonsil, were both widely infiltrated with tumor cells. This was almost the exact location of what was taken to be the primary focus of tumor in case 1.

The meninges covering the medulla, the cerebral peduncles, the third ventricle, the optic tracts and chiasm, and the olfactory bulbs were invaded by tumor cells. Most of the sections from the cortex showed small amounts of tumor in the perivascular spaces, the heaviest infiltration being in the region of the right thalamus.

Sections stained with phosphotungstic acid-hematoxylin, with Hortege's fourth variant for neuroglia and with Cajal's nerve fiber stain, revealed a few fibrils that could be interpreted as neuroglia (fig. 17), and frequent anastomosing cytoplasmic branches typical of neuroglia cells.

Comment.—The tumor was not a sarcoma, not an epithelial tumor and not an endothelial tumor, either in type of cell or in behavior. Its distribution and apparent point of origin bore a striking resemblance to case 1, but cytologically the lesion was composed wholly of undifferentiated cells of the type designated in the clinic as a medulloblastoma.

DISCUSSION OF CASES

The cerebrospinal fluid spaces apparently may become inoculated by malignant cells of various type. The process at least has been variously described as carcinomatosis, sarcomatosis, gliomatosis and, more rarely, endotheliomatosis of the meninges. The literature abounds in descriptions of such lesions though it is often difficult or impossible to determine just what may be the actual nature of the tumor cells the author is describing.¹

With but few exceptions the meningeal involvement in all of the sixty-four cases which have been reported in the literature, has been associated with a definitely recognizable tumor nodule either primarily intracranial or metastatic. Usually both the tumor and the spread of the growth in the meninges were grossly apparent. In a few cases the primary growth was surprisingly small in consideration of the extensive meningeal involvement; in some instances it was never disclosed;² and in the cases here reported it was of microscopic size and only identified after a prolonged search.

Whether a primary and spreading tumefaction which arises *de novo* in the meningeal spaces ever occurs may well be a matter of considerable doubt. Certainly if such a process is possible the only cells which could participate would be the mesothelial cells which line the cerebrospinal fluid channels and play an important rôle as macrophages within

1. A recent article, containing a comprehensive bibliography of the subject with the analysis of seventy-six cases, has recently been written by Oscar Schuberth. Schuberth, Oscar: Ueber diffuse Sarcomatosis und Gliomatosis in den Meningen des zentralen Nervensystems, *Deutsche Ztschr. f. Nervenhe.* **93**:34-58, 1926.

2. This, for example, was true of the case described by Hans Kaiser. Kaiser, Hans: Ueber primäre diffuse Sarcomatose der Leptomeninges des Gehirns und Rückenmarks, *Beitr. z. path. Anat. u. z. allg. Pathol.* **62**:265-284, 1916.

these fluid spaces. It is well known that these latter cells may form the basis of neoplastic formations producing the so-called dural endotheliomas which in this clinic are termed "meningiomas." But these are usually isolated and relatively benign lesions and even though they may infiltrate the meninges locally and may recur after operation they have never been known to infect the meningeal spaces in the manner under discussion. It was for this reason that particular interest was felt in the first case herein reported when the microscope disclosed what might be called a "meningiomatosis" (endotheliomatosis) of the leptomeningeal pathway.

We have been able to identify in the literature only three or four examples of a process corresponding to or comparable with that found in our case 1. One of them from this clinic has been studied in detail recently by B. M. Fried, and recorded³ as an example of true "sarcomatosis"—a term, as should be emphasized, which, for want of proper differential stains, has been heretofore loosely and inaccurately employed. Fried's case and those of Cassirer and Lewy (case 1),⁴ of Markus⁵ and possibly also of Schaede (case 1)⁶ and of Nonne⁷ appear to be the only authentic examples of what would deserve, on histogenetic grounds, the designation sarcomatosis, mesotheliomatosis or meningiomatosis. The similar ways in which the perivascular spaces are primarily affected by the neoplasm in case 1 of this report and in the cases reported by Cassirer and Lewy and by Markus are shown in figures 9, 10 and 11.

Obviously, many of those who in the past have reported examples of meningeal invasion by tumor have had misgivings as to the precise histogenesis of the lesion. It is equally apparent that many of the cases which confuse the literature of the subject might have been shown to be actual gliomatosis, had proper differential stains been successfully employed.

3. Fried, B. M.: Sarcomatosis of the Brain, *Arch. Neurol. & Psychiat.* **15**: 205-17 (Feb.) 1926.

4. Cassirer, R., and Lewy, F. H.: Zwei Fälle von flachen Hirntumoren, *Ztschr. f. d. ges. Neurol. u. Psychiat.* **61**:119-145, 1920.

5. Markus, O.: Ein Fall von diffuser Sarkomatose der Pia Mater, *Arch. f. Psychiat.* **51**:322-33, 1913.

6. Schaede, G.: Ueber diffuse Geschwulstbildung in der Pia Mater, *Ztschr. f. d. ges. Neurol. u. Psychiat.* **6**:96-124, 1911.

7. Nonne, M.: Ueber diffuse Sarkomatose der Pia Mater des ganzen Centralnervensystems, *Deutsche Ztschr. f. Nerven.* **21**:396, 1902. We have doubts of this case, though the author ascribes the tumor to the perithelium of the blood vessels of the pia. He makes the curious statement that "Der subarachnoidal-Raum war überall frei von Neubildung, wie auch das äussere Blatt der Arachnoidia." The paper is important in that it apparently is the first report of a carefully studied case in which a primary tumor nodule was not disclosed.

In a recent article on the subject of "leptomeningeal sarcomatosis" by Ford and Firor,⁸ four personally observed cases are reported, one of them, as mentioned in a footnote, having subsequently been proved to be glioma. Their other three cases, indeed, may be regarded as doubtfully "sarcomatous," as the authors are compelled to admit, no tissues having been taken for examination in one of them.

The impression seems to be gaining ground that the majority of the cases which have been described as sarcomatosis of the meninges are, in reality, gliomas—in other words, that they represent an invasion of the meninges by tumor which has originated in the brain tissues. This

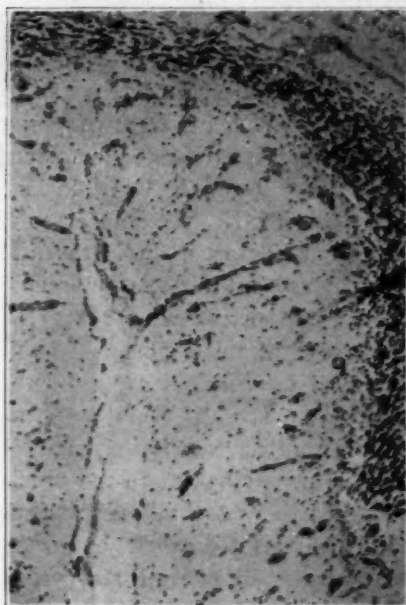


Figure 9

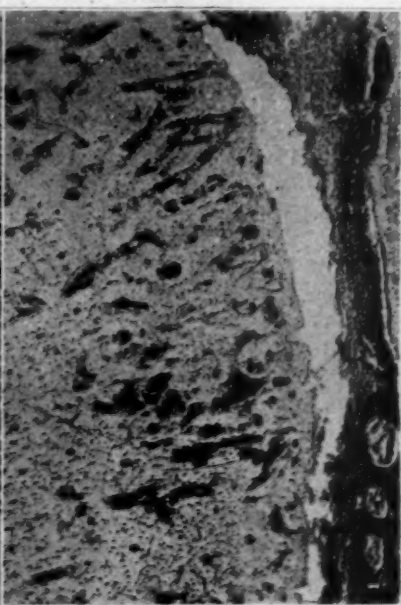


Figure 10

Fig. 9.—The characteristic type of multiple perivascular origin of the neoplasm in our case 1, compared with the case described by Cassirer and Lewy.

Fig. 10.—From the case described by Cassirer and Lewy; comparison should be made with figure 9.

does not necessarily mean that glial fibrils are always to be identified to justify the term meningeal gliomatosis; for certain of the tumors, as has been found to be true, for example, in Spiller's⁹ case, prove to be gliomas of ependymal origin, which should be recognizable by the morphology and arrangement of the cells and by the presence of

8. Ford, F. R., and Firor, W. M.: Primary "Sarcomatosis" of the Leptomeninges, *Bull. Johns Hopkins Hosp.* **35**:65-75, 1924.

9. Spiller, W. G.: Gliomatosis of the Pia and Metastasis of Glioma. *J. Nerv. & Ment. Dis.* **34**:297-302, 1907.

blepharoplasten, as Bailey has made clear in papers dealing with these particular tumors.¹⁰ From the experience of this clinic, however, the ependymomas, although they may reimplant themselves in the cerebrospinal pathway, are much less likely to spread widely through the leptomeninges than a far more common form of glioma, which has been made the subject of special study by Bailey and Cushing¹¹ and called by them medulloblastoma.

These particular tumors, which most commonly occur in childhood and take their origin from the roof of the fourth ventricle, often reach a large size and fail to infect the meningeal spaces even after successive surgical operations resulting in incomplete extirpations. However, they

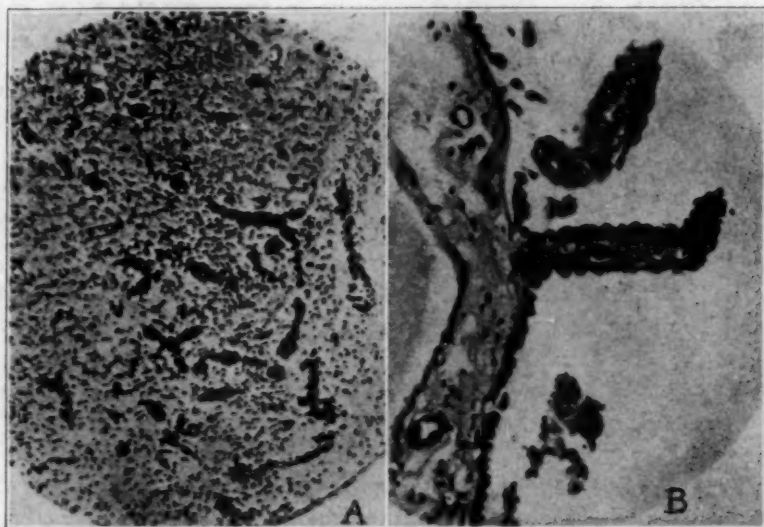


Fig. 11 (from Markus, for comparison with figs. 9 and 10).—Showing (a) the same type of multiple primary perivascular cell accumulations in the cerebellar cortex; (b) similar cells heaped up on the surface and strands of the pia arachnoid.

sometimes do infect the spaces (though rarely so early as in our case 2) with a resultant spread of the tumor either as a diffuse process, or as more or less scattered nodules of the lesion, which become implanted here and there in the pathway of the circulating fluid. Because of the characteristic architecture and the frequent absence of demonstrable

10. Bailey, P.: (1) A Study of Tumors Arising from Ependymal Cells, *Arch. Neurol. & Psychiat.* **11**:1-27 (Jan.) 1924. (2) Quelques nouvelles observations de tumeurs épendymaires, *Ann. d'anat. path.* **2**:481-512 (Nov.) 1925.

11. Bailey, P., and Cushing, H.: Medulloblastoma Cerebelli: a Common Type of Midcerebellar Glioma in Childhood, *Arch. Neurol. & Psychiat.* **14**:192-223 (Aug.) 1925.

glial fibrils, these lesions have in the past often been called neuroblastomas by certain pathologists. The tumors, however, are believed to originate from an anlage of undifferential cells, and since actual nerve elements are rarely demonstrable, an occasional glial cell being much more often found, they may be regarded as potentially glial rather than neural; they certainly are not mesoblastic. The majority of the cases reported in the literature as meningeal sarcomatosis have, therefore, in our opinion, been examples of invasion of the meninges by undifferentiated round cells from a lesion of this sort, which represents a true tumor of the primitive neuroglia and cannot in any sense be regarded as a sarcoma.

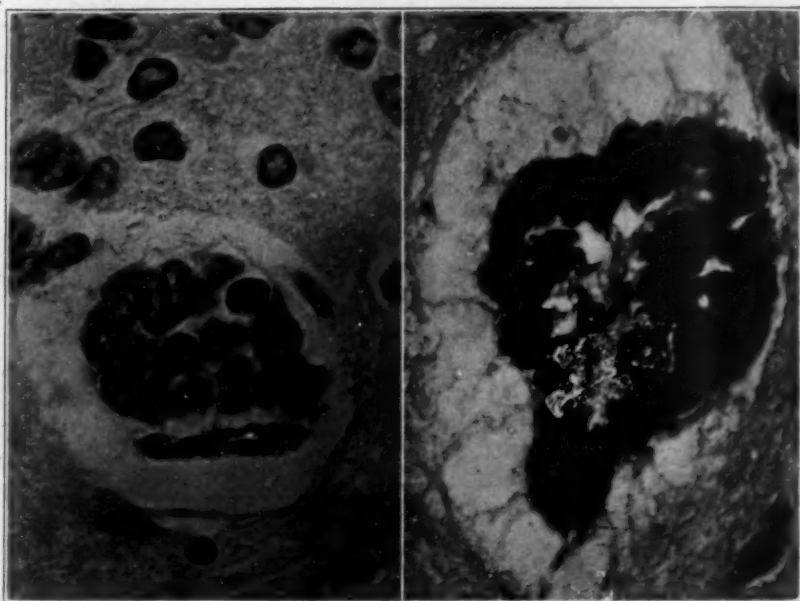


Figure 12

Figure 13

Fig. 12 (case 1).—Showing that the tumor cells form a sort of excrescence from the adventitia of the vessel. Hematoxylin and eosin stain. $\times 850$.

Fig. 13 (case 2).—The tumor cells have infiltrated the normal perivascular sheath of the vessel. Hematoxylin and eosin stain. $\times 850$.

Naturally out of a large number of these tumors there will be some in which the cells have undergone differentiation with the production of glial fibrils. An assemblage of nine examples of this condition of meningeal gliomatosis has been made in a second paper by Firor and Ford,¹² with the inclusion of two personally observed examples; and

12. Firor, W. M., and Ford, F. R.: Gliomatosis of the Leptomeninges, *Bull. Johns Hopkins Hosp.* **35**:108-11, 1924.

Brannan in a more recent article has added still another.¹³ One gains the impression from both of these articles that their authors incline to what is distinctly our own view; namely, that diffuse tumor invasion of the meninges—the so-called “tumor meningitis”—is, in the long run, glial in origin, and that the designation of diffuse sarcomatosis for the process is, in the majority of cases, a misnomer.

Comparative Notes on Our Two Cases.—It is apparent, from even a superficial examination of the two cases which form the basis of this report that, in spite of their similarities in point of origin and manner of spread, they are histogenetically and histologically quite different.

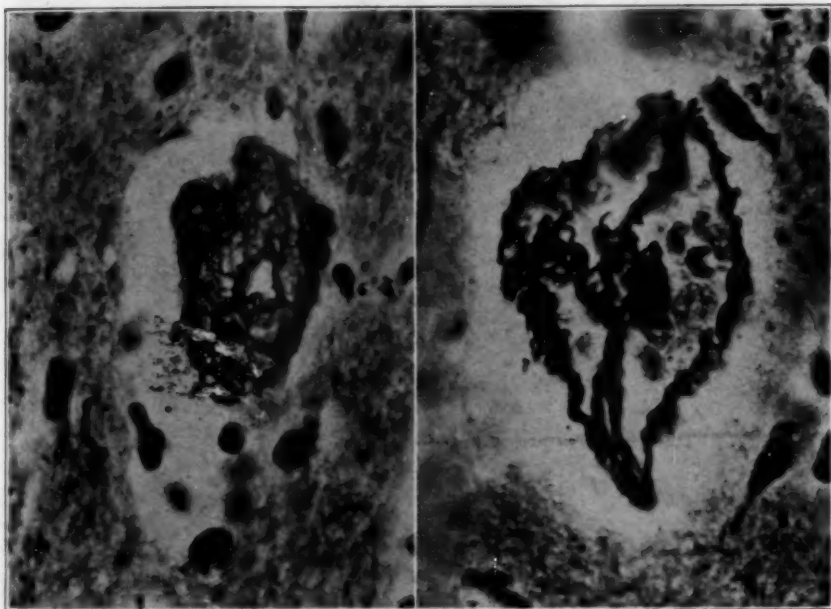


Figure 14

Figure 15

Fig. 14 (case 1).—Each cell is surrounded by reticulum, the whole being an overgrowth of the adventitia. Perdrau's method on Zenker-fixed tissue. $\times 850$.

Fig. 15 (case 2).—The adventitia is normal, the cells lying free in the perivascular space. Perdrau's method. Zenker-fixed tissue. $\times 850$.

In case 1 there is no primary solid mass of tumor in the brain substance, and even where the lesion is most dense (fig. 4) it still retains its perivascular arrangement. In case 2, on the other hand, there is in the same region near the flocculus a solid nodule of tumor, which projects into the ventricle (fig. 8).

13. Brannan, D.: Secondary Gliomatosis of the Leptomeninges, *Am. J. Path.* 2:123-36, 1926.

It is interesting to note that both of these tumors took their origin from the developmentally complicated region near the recess of Luschka. The two areas from which tumors such as peritheliomas, papillomas and medulloblastomas appear most commonly to arise are (1) in the neighborhood of the flocculus at the lateral outlets of the fourth ventricle and (2) in the midline just over the calamus at the foramen of Magendie—areas whose complicated structure might possibly favor developmental anomalies.

The first tumor is unmistakably a primary overgrowth of the adventitial cells of the vessels nourishing the granular layer of the inferior

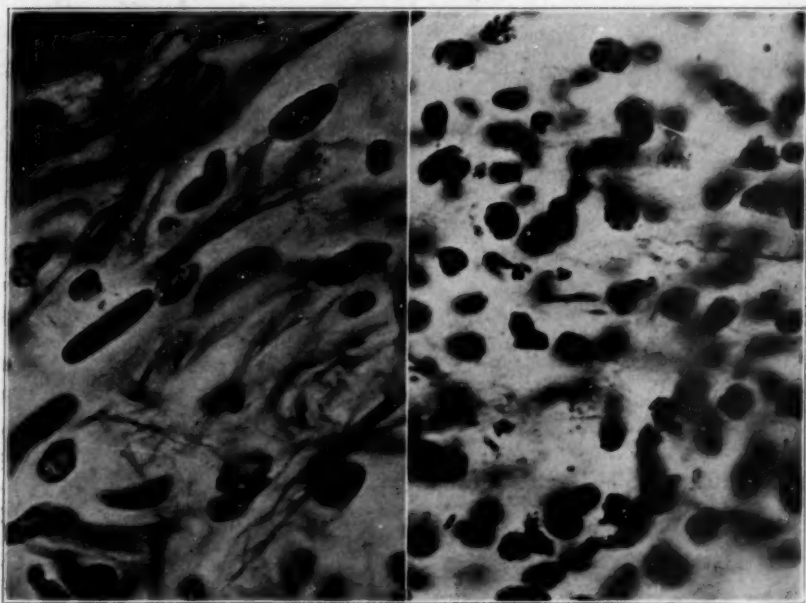


Figure 16

Figure 17

Fig. 16 (case 1).—Elongated cells intimately intermingled with fibrils of collagen and reticulum, probably also fibroglia. Hortega's fourth variant. $\times 850$.

Fig. 17 (case 2).—Rounded cells with delicate cytoplasmic processes rarely heavily impregnated. Hortega's fourth variant. $\times 850$.

folia of the flocculus and is not a secondary involvement of the perivascular spaces, from without inward. On the contrary, the meningeal involvement would appear to be either coincidental or secondary.

In the second tumor, on the other hand, an early meningeal infection by tumor cells has led to a secondary retrograde infiltration of the perivascular spaces.

For purposes of comparison, sections taken across the perivascular spaces in the two tumors may be put side by side (figs. 12 and 13). In figure 12, from case 1, the tumor cells may be seen taking their origin

as a nodule from the adventitia of the blood vessel. In figure 13, from case 2, there is unquestionably a perivascular retrograde invasion from the cerebrospinal spaces inward.

The difference, not only in the relation of the tumor cells to vessel but also in the nature of the tumor cells themselves, is better shown by Perdrau's method. Thus in figure 14 each cell is seen to be surrounded by reticulum, which advocates its adventitial origin, whereas in figure 15, from case 2, the cells do not have any reticulum and the adventitia is normal.

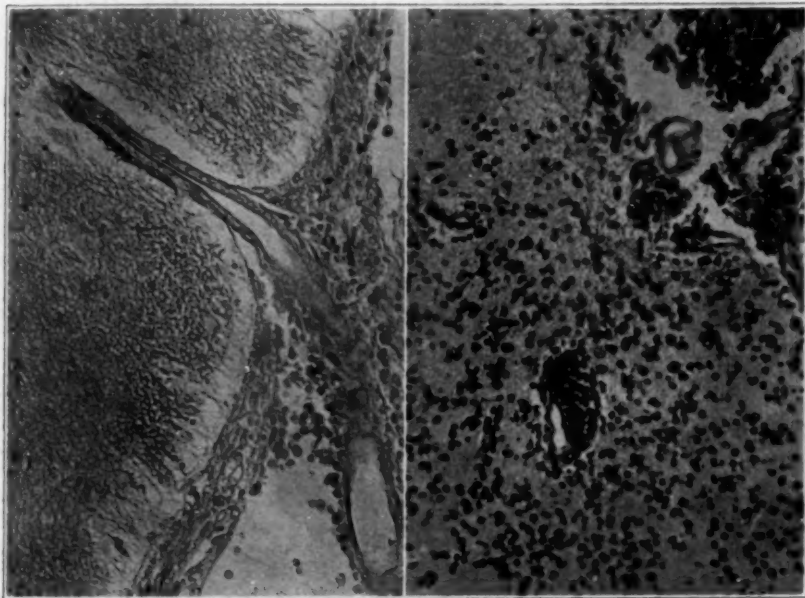


Figure 18

Figure 19

Fig. 18 (case 1).—The tumor stops where the intima pia becomes perithelium. Methylene blue eosin. $\times 150$.

Fig. 19 (case 2).—Tumor spreading from meninges along perivascular space and breaking out of this to spread diffusely in cerebral tissue. Hematoxylin and eosin stain. $\times 150$.

The diffuse tumor in the meninges is also quite different in the two cases. The first tumor (fig. 16) is composed largely of elongated cells with sausage-shaped nuclei, there being numerous collagenous and reticular fibrils between the cells and intimately associated with them.

In contrast, the second tumor (fig. 17) is composed of rounded cells with small heavily staining nuclei with scanty cytoplasm. Only occasionally does one see delicate cytoplasmic processes, a few of which can be stained by phosphotungstic acid-hematoxylin or by Hortege's fourth

variant. In this case, collagen and reticulum can be identified only in the walls of the blood vessels and in the large strands of the leptomeninges.

Over the cerebral cortex also the two tumors behave quite differently. The first tumor (fig. 18) does not secondarily penetrate into the perivascular spaces, though the overlying meninges may be heavily infected. On the contrary, the cells of the second tumor (fig. 19) not only do so penetrate into the perivascular spaces, but also may be seen even to break out of them, so as to spread diffusely in the cerebral substance.

In the first case, in short, it is evident that the tumefaction of the adventitial cells of the vessels (the perithelium of Eberth or the perivascular sheath of pia arachnoid) occurs in multiple foci independently, and is found in areas apart from the diffuse meningeal involvement, which in all likelihood has originated from some one of these perivascular foci, a condition to which Cassirer and Lewy called attention. In the second case the tumor represents a small primary glioma composed of undifferentiated cells allied to the medulloblasts which form the nervous tissue. Early in its growth this tumor has broken into and infected the meninges, the cells having secondarily backed up in many places into the perivascular spaces connected with the cerebrospinal fluid pathway.

SUMMARY

1. Two diffuse tumors of the meninges which differed greatly in their microscopic structure have been described. In neither case was a primary tumor visible to the naked eye, and without microscopic examination both might have been recorded as examples of pseudotumor cerebri.

2. The first of these tumors represents a true sarcomatosis (endotheliomatosis) of the meninges, the primary lesion apparently being multiple and affecting the adventitial or perivascular sheaths of the cortical blood vessels. Exceedingly few examples of a tumor of this nature have been described clearly.

3. The second tumor represents a far more common process, and represents an invasion of the leptomeningeal spaces by largely undifferentiated embryonic cells (medulloblasts). As these primitive round cells, commonly miscalled sarcoma cells, tend to undergo a certain degree of differentiation and when properly stained show occasional neuroglial fibrils, a condition deserving the designation meningiogliomatosis is the consequence.

4. The primary origin of both of these tumors was in the region of the flocculus, an area which appears to be a favorite point of origin for neoplastic lesions.

FEVER WITH RENAL CARCINOMA *

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Fever is not generally regarded as a symptom of renal tumor. If a patient with renal or abdominal symptoms has fever, that sign usually removes early new growth from the differential diagnosis. This is contrary to fact, because there is abundant evidence that fever does occur in uncomplicated new growth, although not as frequently as in inflammatory disease.

The occurrence of fever in malignant disease was the subject of a recent article by Briggs,¹ in which he not only reviewed medical records, but also analyzed 238 of his own cases, comprising various types of malignancy in every part of the body. Fever was present in 38 per cent of his own cases, and in 45 per cent of all cases in which ulceration of the new growth had taken place. In his article, ulceration of a new growth was classed as a complication. No mention was made of kidney tumors in the original observations, but there was a six line reference to an article by Marsh² on fever in two cases of sarcoma, one of the kidney and the other of the femur.

The most instructive reference on this subject is given by Israel,³ who quoted figures from 146 cases of kidney and suprarenal tumors, which he removed by surgical operation. Of the uncomplicated cases, fever was present in 8 per cent. In his discussion as to the cause he says, "One does not yet know what the fever-exciting, noxious agent is. Degeneration resulting from bacteria action, which readily plays a rôle in stomach, bowel, and uterine tumors, is out of the question in the wholly aseptic tumors, of the kidney and adrenal. Necrobiosis which occurs in all malignant tumors, should not bear the burden because fever is not always present. The cause of the fever must be looked for either in the rapid growth of the tumor cells or the destruction of normal tissue. The first is for many reasons the more probable." The temperature fell to normal in five cases when the tumor was removed. In some of the others, it remained above normal because metastases appeared. According to Israel, all these facts leave no doubt that the fever

* From the Departments of Pathology of the University of Manitoba and the Winnipeg General Hospital.

1. Briggs, L. H.: The Occurrence of Fever in Malignant Disease, *Am. J. M. Sc.* **166**:846 (Dec.) 1923.

2. Marsh, H.: *St. Bartholomew's Hosp. Rep.* **23**:147, 1887.

3. Israel, J.: *Fieber bei malignen Nieren und Nebennierentumoren*, Freie Vereinigung der Chirurgen Berlins, 189 Sitzung, den 14 Nov., 1910, *Zentralbl. f. Chir.* **41**:10, 1911.

depended on the new growth. He did not differentiate the various types of malignant kidney tumors on which these observations were made.

Voelcker⁴ said that fever was not infrequently observed in malignant hypernephromas. It often lasted a week, with evening rises of from 100.5 to 102 F. Cases are known in which fever was the first recognizable feature of the illness, so that it had the character of an early sign. In speculating as to the cause, Voelcker refers to the absorption of albumin bodies produced by the process of involution. He also mentioned as a possibility that inflammation of the pelvis of the kidney plays a rôle, because this structure is often invaded by plugs of necrotic new growth.

Great size and the rapid growth of hypernephromas were factors associated with fever, according to the observations of Berg.⁵

The frequency of fever associated with hypernephromas was the subject of a recent article by Castaño.⁶ Of his series of seven kidney tumors, six were hypernephromas. In three of the cases, there had not been any symptoms from the urinary tract; chills were present in two, and irregular fever alone was present in one. The fever, intermittent in type, appeared early and would be the only symptom for a time. In each case it subsided after nephrectomy.

All these observations afford ample proof that fever occurs in uncomplicated kidney tumors, but the following case is cited to illustrate how, when no palpable mass is present, an increase of temperature may obscure the diagnosis of renal neoplasm.

REPORT OF CASE

History.—A trained nurse, aged 38, had always enjoyed good health with the exception of having had a gastric disorder at the age of 20. This complaint was diagnosed as gastric ulcer. A gastro-enterostomy was performed, and after about a year's illness, the patient recovered. She was of the angular type, tall and thin.

In the fall of 1918, following hard work at military nursing, an osteo-arthritis of the tarsal bones developed. As treatment for this in March, 1919, an arthrodesis was performed, and plaster casts were applied. During her two months' stay in the hospital, there was not any fever.

In March, 1920, she suffered from discomfort in the abdomen for two or three days. This suddenly developed into a severe pain which was not relieved by hot applications. The temperature rose to 104 F., and afternoon elevations continued for two weeks. There was marked tenderness, particularly on the right side. She recovered after three weeks' illness.

4. Voelcker, F.: Die Neubildungen der Niere, in Kraus and Brugsch: *Spezielle Pathologie und Therapie inner Krankheiten*, Berlin, Urban & Schwarzenberg, 1920, vol. 7, p. 653.

5. Berg, A. A.: Malignant Hypernephroma of the Kidney, Its Clinical Course and Diagnosis, with a Description of the Author's Method of Radical Operative Cure, *Surg. Gynec. Obst.* **17**:463-71 (Oct.) 1913.

6. Castaño, C. A., and Risolia, A. J.: Kidney Tumors, *Semana méd.* **1**:989 (May 24) 1923 abstr., *J. A. M. A.* **81**:1730 (Nov. 17) 1923.

The next illness was in September, 1920, when she complained of headaches, a metallic taste in the mouth, urinary frequency and pain in the right loin. Examination showed a systolic murmur at the cardiac apex, the lungs clear, some epigastric tenderness but no mass. The temperature ranged from 99 to 101 F.

Examination.—During October, a thorough examination was made. The heart, lungs, pelvis, cystoscopic and pyelographic examinations gave negative results. All laboratory tests gave negative reactions except one urinalysis, which showed numerous red blood cells. The phenolsulphonphthalein dye excretion test was only 22 per cent in two hours. One day only 15 ounces (444 mg.) of urine were passed. The daily temperature was above 100 F. in the afternoons, and she had vague pains in the shoulders, knees and toes.

Treatment and Course.—In November, weakness and dizziness came on. Pain in the right loin was moderate, but there was no tenderness. A rough systolic murmur was now noticed. The first sound was encroached on but no distinct presystolic murmur was present. A provisional diagnosis of subacute endocarditis was made. In two weeks the patient was feeling better. The heart sounds were vigorous and the murmur slight. There was not any cough or sputum. The urine output varied from 16 to 30 ounces (473 to 887 mg.) daily. Slight but constant tenderness was present on deep pressure just below the right costal margin. After deep inspiration the bulk of the right kidney seemed increased and displaced downward, but the tenderness described was above and nearer the midline. For this reason, the gallbladder was suggested as the origin, although the proximity to the aorta may have accounted for the sensitiveness.

In February, after eleven months of suffering from abdominal pain and general weakness, the roentgen ray revealed a broadening of the hilum shadows of the lungs. Careful examinations had previously failed to reveal any cause of the illness. She now began to feel exhausted and generally ill, although she kept going about. An afternoon temperature of from 100 to 102 F. was an almost daily occurrence.

In September she again entered the hospital, complaining of severe headache and pain in the right shoulder and in the chest. For eighteen months, she had had an afternoon fever almost daily. Roentgen-ray examination now showed an increase in the hilum shadows and a number of annular opaque areas in the chest, particularly in the right side. A diagnosis of Hodgkin's disease was made, and roentgen-ray treatment was given, but without benefit.

By November a moderate degree of secondary anemia had developed, and she received a blood transfusion. Weekly attacks of abdominal pain occurred which could be controlled only by morphia. After November, her condition rapidly became worse. She complained of intense abdominal pain and inability to void urine at times. She was troubled with falling sensations and dizziness. Occasionally the extremities became cold and clammy. On January 11, she died. There had been no increase in temperature for the last forty days of her illness.

Clinical Summary.—Almost continuous fever, with intermittent abdominal pain and general weakness in a woman, aged 38, remained undiagnosed for eleven months, in spite of careful examination. Roentgen-ray examination then showed a widening of the lung hilum. Seven months later, eighteen months after the onset of the illness, annular opaque areas appeared in the lung and a diagnosis of lung tumor, probably Hodgkin's disease, was made. Fever had been present almost continuously for twenty months.

Autopsy.—Autopsy showed an emaciated body with some edema about the ankles and feet.

Opening of the abdominal cavity revealed that the omentum was puckered in a knot and adherent to the underlying structures 5 cm. above and to the right of the umbilicus. When the transverse colon was lifted, the mesentery of the small intestine was seen to be adherent to the posterior wall of the stomach, 7 cm. from the pylorus. No portion of the small intestine adhered to the stomach, however. A small linear scar 2 cm. in length and parallel to the long axis of the body was seen on the mucosa of the posterior wall of the stomach at the point where the mesentery of the jejunum was attached externally. This was the site of an ulcer excision or a gastro-enterostomy that had closed up. The liver showed a moderate amount of congestion. Several small yellowish tumor nodules were seen, mostly

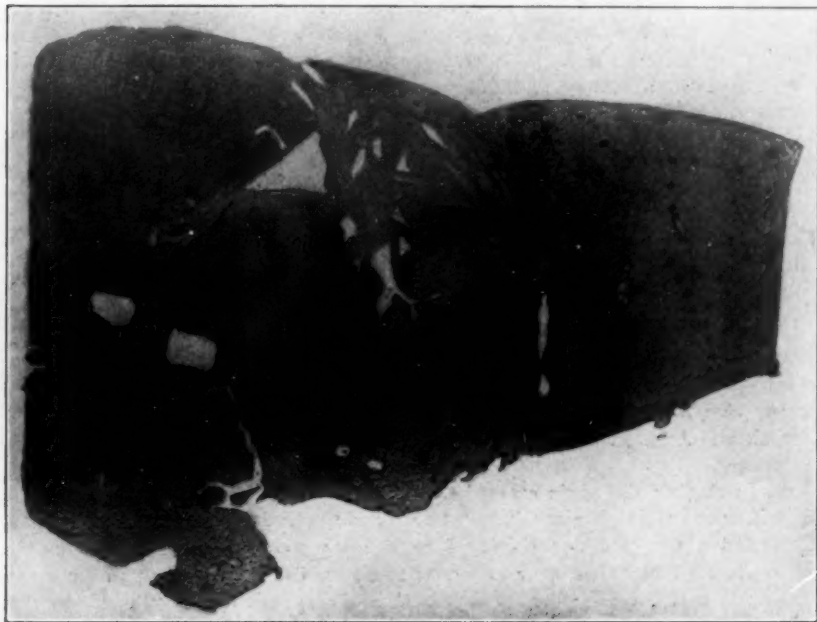


Fig. 1.—Section showing kidney substance to the right separated from tumor to the left by a false capsule which contains the remains of glomeruli. Magnification, 7 diameters. Contents of circle at the left are more highly magnified in figure 2.

near the surface of the organ. A small, subserous, yellowish nodule, similar in appearance to those in the liver, was seen on the inferior border of the spleen.

The right kidney was difficult to remove and its lower pole contained a large tumor 15 cm. in diameter, of mottled appearance, grayish and translucent, bright yellow, reddish and dark brown. Longitudinal section showed that this tumor extended from the cortex to the pelvis of the kidney.

Microscopic sections showed a most unusual picture. A homogeneous, small cell tumor contained nodules of a large cell papillary structure. This gave the appearance of a large cell tumor within a small cell one. Many large, irregularly shaped, thin walled blood vessels were present, and at intervals downgrowths of fibrous tissue dipped in from the margin which was quite circumscribed.

The kidney tissue was much compressed so that the normal structure was distorted or fibrosed to form a false capsule which contained the remains of some glomeruli (fig. 1).

The main, small cell tumor was made up of cells arranged in a diffuse fashion, but in places some poorly formed acini were seen. The nuclei were round, deeply stained and smaller than lymphocytes. The cytoplasm had a diffuse, myxomatous appearance, and only near the large cell areas could the cell outlines be distinguished.



Fig. 2.—Section of kidney tumor showing small cell part above and a large cell nodule below, with its papillary form and an area of degeneration. Magnification, 75 diameters.

The large cell nodules within the tumor presented a striking contrast. Its large cells, with cytoplasm of clear or faint cobweb appearance and nuclei two or three times as large as those of the other part of the tumor, were arranged in irregular papillae, sometimes as acini and rarely in rows. Sometimes the structure was replaced in the center of the nodule by diffuse colloid products of degeneration staining pink, with an irregular granular material of blue black, probably nuclear remains (fig. 2).

The remaining part of the kidney showed only slight change, such as cloudy swelling of the convoluted tubules, distention of the blood vessels and exudate into an occasional glomerular space.

The left kidney did not show any pathologic change, but it was somewhat larger than normal, and weighed 250 Gm.

The secondary deposits in the other organs showed the same structure under the microscope as the one already described in the kidney. In the left suprarenal there was a nodule 1.5 cm. in diameter, with a gross appearance similar to that of the other tumor nodules. The contrast between the cells of the tumor nodule in the suprarenal and those of the suprarenal cortex was marked. The tumor cells were irregular in both size and grouping, sometimes in acini and again in rows, but they were always twice the size of the cells of the suprarenal cortex.

The intestines, pancreas and pelvic organs were normal.

The anterior borders of the lungs appeared normal, but on the right side a nodule about 2 cm. in diameter was seen bulging above the surface of the middle lobe. It was similar in microscopic appearance to the nodules found in the kidney. The posterior part of the lung was dark and confluent. The upper lobe of the left lung contained three nodules similar in appearance to, but not so large as, those described in the right lung.

The pericardial sac was normal. Beyond an unusual degree of pallor the myocardium did not show any pathologic change. The mitral ring, however, readily admitted the tips of three fingers. The aorta was normal.

The bone marrow was normal. The cranial contents showed a moderate edema over the cerebral hemispheres.

COMMENT

There has been much disagreement as to whether such a tumor as this is a renal carcinoma or a hypernephroma. Wright,⁷ in a comprehensive article, describes such tumors under the head of hypernephromas. He summarizes the views on the origin of such tumors up to the present. From his own observations, he stresses the papillary microscopic appearance and regards the growth as a result of malignant change in the renal tubules and therefore as a carcinomatous lesion.

Ewing⁸ classifies as adenocarcinoma tumors that have clear cells in a papillary or acinar arrangement and as hypernephromas, tumors that are the exact reproduction of benign suprarenal growth, with added mesoblastic tendencies, a fibrous core and chromaffin cells at times. In this tumor, the tendency of the small cell part to form acini in some areas and the papillary arrangement of the large cell part would make carcinoma the most likely diagnosis.

SUMMARY

1. A detailed history is given of a patient who suffered from gastrointestinal and urinary disturbances, with vague neuralgic pains, for a period of about two years.

7. Wright, H. W. S.: Study of the Surgical Pathology of Hypernephromata: Origin and Symptomatology, *Brit. J. Surg.* 9:338-365 (Jan.) 1922.

8. Ewing, J.: *Neoplastic Diseases*, ed. 2, Philadelphia, W. B. Saunders Company, 1922, p. 761.

2. Fever was one of the most marked signs and was present almost continuously.

3. Autopsy showed a renal carcinoma of the lower pole of the right kidney with secondary growths in the left suprarenal, spleen, liver and lungs.

4. Some parts of the tumors were made up of small cells which tended to form alveoli; other parts were made up of large clear cells which formed papillae, or irregular elongated acini, and at times showed hyaline degeneration.

ANTEROMEDIAN MERO-ACRANIA (NOSENCEPHALOS)
COMBINED WITH HYPOGASTROSCHISIS DEXTRA AND MALFORMATION
OF FINGERS AND TOES: REPORT OF A CASE

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CHICAGO

In the male fetus of a primipara, aged 19, the length of the cord was 3 inches (7.6 cm.); the length of the fetus, 13 inches (33 cm.); the height of the trunk, 7 inches (18 cm.), and the circumference of the chest, 6 inches (15 cm.).



Fig. 1.—Front view of fetus.

The appearance of the skin was normal; the lanugo and hairs of the head were well developed. The vault of the head was not closed, and the brain was expelled during delivery. The parietal and frontal bones were absent. The facial part of the skull presented an open cavity. The face consisted of the upper maxillary arch and mandible. The upper palate was well developed; the tongue was present. The meatus auditorius externus sinister was open; behind



Fig. 2.—Back view of fetus.

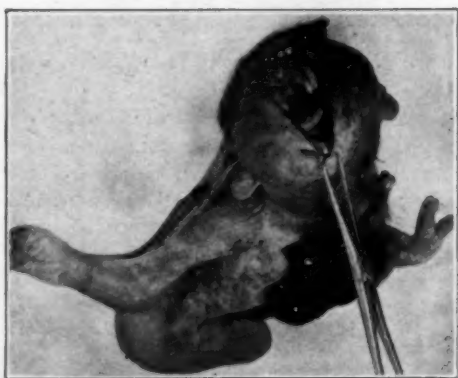


Fig. 3.—Face with tongue.

the latter the external ear was overdeveloped with an adhesion stretching from the upper part of the deltoid region of brachium. The right ear was overdeveloped and fairly normal. There was a sharp bony projection in the region of the left eye. The left eye presented a small aperture with an overdeveloped upper lid and no lower lid. The region of the right eye was covered by epidermal tissue. The maxilla was hypertrophic; there was no cleft palate and no lips; the dorsal part of the wall of the palate was everted anteriorly. The thyroid and cricoid cartilages were well developed. There was a skin appendage on the neck, about 1 inch (2.5 cm.) in length.

The right side of the abdominal wall was open. The liver, small intestines and right kidney were extra-abdominal. The sternum and ribs on the left side were well developed; beginning from the vertebral column, the right side was curved markedly caudally (fig. 4). The right forearm was bent backward; the



Fig. 4.—Roentgenogram of fetus.

hand had only two fingers and both were overdeveloped. The left scapula was present, the left arm was well developed and from the middle of the arm there was an adhesion to the left ear. The adhesion consisted of a cord of skin. The left hand had three fingers, all overdeveloped; the thumb and middle finger were connected, and the middle finger was long and thick.

The opening of the abdominal wall was 5 cm. in length, the umbilicus was open, and the abdominal organs (liver, kidney, gallbladder, small intestine) were everted at the right side and extraperitoneal; the transverse colon was visible and covered by peritoneum. The penis was overdeveloped, and the testicles were not descended.

The length of the right thigh was 2 inches (5 cm.); the left thigh, $2\frac{1}{2}$ inches (6.2 cm.); the right leg, 2 inches (5 cm.); the left leg, $2\frac{1}{2}$ inches (6.2 cm.). The right foot had the shape of a clubfoot and was inverted and adducted; there were four toes—the first and second were firmly connected. The left foot had a nor-

mal position; it had five toes, and four of them were enveloped by a common skin and had no end phalanges. The fifth presented all the phalanges and was covered by a nail.

COMMENT

There were three different monstrosities in the fetus: cranioschisis, hypogastroschisis and malformations of the extremities. The cranioschisis was of the anteromedian type: both the parietal and the frontal bones were almost entirely lacking. The hypogastroschisis connected with a partial thoraco-omphaloschisis was continued into the chest, but only as far as the skin was patent; the lungs and ribs were compressed by the eventration of the hypogastric organs, caused by the arrest of the closure of the lateral abdominal wall. There was another resemblance to a thoraco-omphaloschisis—that was the fixation of the right arm to the thoracic wall. Similar cases have been reported by Ballantyne.¹

The underdevelopment of the right thigh and right leg may have been the consequence of the diminished blood supply caused by the eventration of the abdominal organs.

Both the cranioschisis and the hypogastroschisis were of ectodermal origin, probably early in the embryonic life. The malformation of the fingers and toes is reported in many cases of acranias, but the determining factors are not yet known.

The shortness of the cord may have been the cause of the wrong development during fetal life (between the second and third weeks); but it is not easy in this case to separate the causes from the effects.

1. Ballantyne: *Manual of Antenatal Pathology: The Embryo*, New York, William Wood & Co., 1905.

ATHEROMATOUS DEGENERATION OF THE ARTERIAL WALL

THE RESULT OF A HYDRODYNAMIC MECHANISM *

V. H. MOON, M.D.

INDIANAPOLIS

A mathematical application of the principles of dynamics is seldom attempted in explaining pathologic processes. This is due to the fact that fewer opportunities for such application are offered in pathology than in other branches of science. When the problem is appropriate for a combination of mathematical and physical analysis, the resulting conclusions should be as trustworthy as those based on an experimental study of disease. Some of the factors concerned in the circulation of blood through the vasa vasorum are hydrodynamic in character and invite an attempt at such analysis.

It is taught that the walls of the large arteries are supplied with blood through the vasa vasorum located in the adventitia and distributing to capillaries in the media and intima. The blood pressure in these capillaries has not been measured, but it may be assumed to approximate that of other capillaries which is about one-sixth of the diastolic blood pressure. Some of the capillaries nearest the lining of the artery are only a small fraction of a millimeter distant from a surface which continually receives a pressure much higher than that within the capillaries. Should the external pressure exerted on these capillaries exceed the pressure of blood flowing within them, they would be collapsed and could no longer carry blood. The capillaries are separated from that surface by an elastic structure which absorbs much of the pressure of blood exerted against the arterial lining. The pressure so lost by transmission through successive layers of the artery wall may be spoken of as absorbed by "tissue resistance." In a condition of hypertension, the increased arterial blood pressure results in a slight increase in capillary blood pressure. A large fraction of the increment in arterial blood pressure is lost by friction, stream bed resistance, etc., so that the resulting increment in capillary pressure is relatively small. For the sake of convenience the following symbols will be used: *AP* indicates the normal minimal or diastolic blood pressure in an artery; *CP*, the capillary blood pressure in the wall of that artery; *TR*, the tissue resistance mentioned above; *I*, the increment of arterial blood pressure in hypertension; *i*, the increment of capillary blood pressure in hypertension; *D*, the distance radially from the artery lining toward adventitia.

* From the Department of Pathology, Indiana University School of Medicine

In developing the proposition, it is necessary that the following premises be granted:

1. Fluid may flow through a collapsible tube only so long as the fluid pressure within the tube is equal to or greater than the sum of the pressures exerted externally on that collapsible tube.

2. Blood pressure in the capillaries of the vasa vasorum of an artery is less than diastolic blood pressure in that artery: $CP < AP$.

3. In hypertension the increment in capillary blood pressure is less than the increment of arterial blood pressure: $i < I$.

4. Circulation of blood through the capillaries of the vasa vasorum normally occurs except in the intimal portion of the artery wall.

AUTHOR'S APPLICATION OF PRINCIPLE OF DYNAMICS

Tissue resistance, TR , is not constant for all depths in the artery wall. It varies directly with D or with some function of D . Let $D, D', D'',$ etc., indicate different distances into the artery wall beginning at the lining surface, and let $T, TR', TR'',$ etc., indicate the corresponding tissue resistance at these depths.

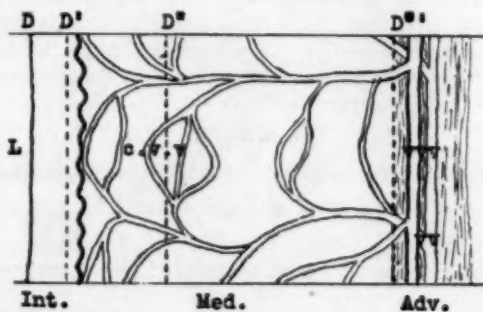


Fig. 1.—Diagrammatic representation of section through artery wall; L indicates lumen; *Int.*, intima; *Med.*, media; *Adv.*, adventitia; *v.v.*, vasa vasorum; *c.v.v.*, capillaries of vasa vasorum.

Since it has been granted in premise 4 that blood circulates through the capillaries of the vasa vasorum, the following condition may be stated mathematically as applying to a plane in the outer portion of the artery wall a distance of D'' from the lining surface:

1. $CP > AP - TR''$, otherwise premise 1 would be violated.

At the lining surface where D equals 0, TR also equals 0; hence by premise 2:

2. $CP < AP - TR$.

Now let D approach D'' , TR approaching TR'' , and a plane will be reached as D' where:

3. $CP = AP - TR'$.

Then at this plane, distant from the lining by D' , circulation through the capillaries is possible; between this plane and the lining of the artery capillary circulation is not possible (premise 1). It is taught that there is no capillary circulation in the portion of the artery wall corresponding to this area in the

diagram. The tissue in this area is said to receive its circulatory nutriment directly from the blood flowing within the artery.

Now let the values of CP and AP be changed to a condition of hypertension, and increments represented by i and I be added to them. Then, since $i < I$ (premise 3), equation 3 will become:

$$4. CP + i < AP - TR' + I.$$

Where this inequality holds, there can be no circulation through the capillaries. The effect of the hypertension has been to produce pressure anemia in this zone in which under normal pressures capillary circulation was possible.

Again let D' approach D'' , TR' likewise approaching TR'' , and a plane will be reached as D'' in which:

$$5. CP + i = AP - TR'' + I.$$

At this plane, circulation through the capillaries will be possible under the altered pressures of hypertension. But the zone of artery wall lying between the distances D'' and D' has been rendered anemic by the increase in arterial pressure. How wide this zone may be will depend on what values may be assigned to i and I , or in other words, on the degree of the hypertension. Whether degeneration and necrosis of the tissues of the wall will occur will depend partly on the duration of the altered blood pressure, and partly on the depth to which oxygen and other nutriment from the blood flowing through the artery may permeate sufficiently to maintain cellular life. If beyond this depth the pressure anemia is of long duration, the resulting degeneration and subsequent atheromatous changes do not need further elucidation.

COMMENT

Many observed occurrences support the hypothesis that atheromatous degeneration begins as an area of local anemia which may result from increased blood pressure:

Atheromatous degeneration is associated frequently with increased arterial pressure.

The cells in the areas affected proceed through the successive stages of swelling, fatty infiltration, necrosis and calcification, independent of demonstrable metabolic, chemical or bacterial injury, as do areas elsewhere when rendered anemic.

The fact that the degenerated area in its early stages is regularly covered by an intact endothelial lining supported by a thin layer of unchanged elastic tissue coincides with the foregoing hypothesis. The portion of the intima nearest the lumen, receiving its nutritional needs from the arterial blood stream flowing through that lumen, is not affected by pressure anemia as are the deeper portions of the wall.

A time-honored explanation of the mechanism of atheromatous degeneration was that the areas involved suffered anemia due to endarteritis or other obstruction of the vasa vasorum. Such obstruction of these arteries was not demonstrable with sufficient regularity to establish this as the probable mechanism. This explanation, however, took cognizance of the features in which atheromatous degeneration resembles the changes which would be expected to follow local anemia in the vessel wall.

The areas in which atheromatous degeneration makes its earliest appearance are those most subject to arterial hypertension:

It has been noted that in congenital constriction of the aorta in young persons, atheromatous degeneration may occur proximal to the stenosis, not distal to it. Aortic narrowing would cause hypertension in the portion proximal to the stenosis.

In cardiac conditions resulting in hypertension in the pulmonary arteries those arteries frequently show atheromatous degeneration. I have not found any account of such degeneration except in patients who had pulmonary hypertension.

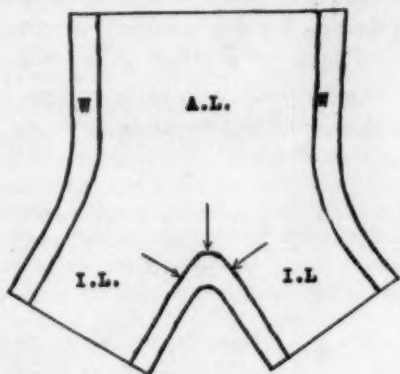


Fig. 2.—Section at bifurcation of aorta; *W* indicates artery wall; *A.L.*, aortic lumen; *I.L.*, iliac lumen; the portion of the intima between the arrows receives hydraulic pressure from three directions.

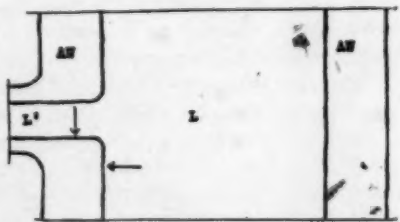


Fig. 3.—Section of an artery at the point of origin of a smaller artery; *AW* indicates artery wall; *L*, lumen of large artery; *L'*, lumen of smaller artery; the portion of intima between the arrows receives pressure from two sides.

The occurrence of degeneration at the bifurcation of the aorta and in narrow zones immediately surrounding the orifices of arteries leaving the aorta is characteristic of the earliest atheromatous degeneration. These are areas in which minimal degrees of hypertension would exert maximal pressure effects, because in these areas such pressure would be exerted on the wall from two directions. Hence slight hypertension might cause local anemia of the artery wall earlier than in areas in which

the pressure would be exerted from only one direction (figs. 2 and 3). The early occurrence of atheroma in these areas has received frequent comment, and has been interpreted as due to strain or increased tension, but the mechanism by which strain or hypertension might cause the degeneration was not shown.

Evidence has been obtained by animal experimentation tending to show that increased arterial blood pressure will produce atheromatous degeneration of the arteries, but again the mechanism of that result was not shown.

CONCLUSIONS

The proposition that arterial hypertension produces local anemia in the artery wall is capable of a mathematical demonstration. The local anemia thus produced may be a cause of atheromatous degeneration. This explanation is in agreement with many known facts concerning the occurrence and course of such degeneration.

Laboratory and Technical Notes

A RAPID CLINICAL METHOD FOR THE DETERMINATION OF THE ICTERUS INDEX*

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The clinical value of any laboratory test depends largely on its simplicity and availability. In working with the icterus index,¹ the following procedure has been found to give satisfactory and sufficiently accurate results for clinical purposes.

The test consists primarily of comparing the color of the blood plasma or serum with a series of permanent arbitrary standards. To obtain the specimen, from 3 to 5 cc. of blood is withdrawn with a dry sterile syringe and placed in a dry sterile centrifuge tube. The blood must not come in contact with any hemolytic agent, such as water, alcohol or ether. If the reading is desired at once, the specimen is centrifugalized at low speed for two minutes and the serum pipetted off. If the determination is not to be made until the following day, the tube is placed in the icebox, where the serum will separate naturally. Slight opalescence, due to the constituents of the blood plasma, does not interfere with the color comparison, but may be avoided if the blood sample is withdrawn several hours after the last meal. Slight hemolysis does not vitiate the test.

The plasma obtained by centrifugalizing oxalated blood, collected for chemical blood analysis, may also be used.

The unit consists of a solution suggested by Meulengracht,² and contains 0.05 parts of potassium bichromate in 500 parts of distilled water, to which 2 drops of concentrated sulphuric acid are added. This gives an index of 1.

The standards consist of a series of fifteen tubes containing multiples of the unit color, the strength of the color being etched on tube. A solution of chemically pure biochromate of potassium is prepared, using 0.15 Gm. in 100 cc. of water and 2 drops of concentrated sulphuric acid; a series of dilutions with distilled water, fourteen lower standards, are also prepared. For example, the next standard is made by using fourteen parts of the original solution and one part of distilled water, the second with thirteen parts of the original solution and two parts of distilled water, and so on until the unit solution is made with one part of the original solution and fourteen parts of distilled water. This solution, kept in air-tight containers, is permanent and has the color of a pure bilirubin solution. The permanent standards are placed in white glass tubes of uniform bore and thickness, filled two-thirds full and sealed.

An identical tube is graduated with six 0.5 cc. marks; 0.5 cc. of the serum to be examined is placed in this tube. The degree of color of this sample is determined by matching it with the standards which are placed on each side of the specimen tube and examined through small holes against a background of ground glass. The number of the tube which approximates that of the sample

* From the Pathological Department, Bellevue and Allied Hospitals.

1. St. George, A. V., and Brown, A. L.: The Value of the Icterus Index in Differentiating Anemia, *Arch. Int. Med.* **36**:847 (Dec.) 1925.

2. Meulengracht: *Deutsches Arch. f. klin. Med.* **137**:38, 1921.

is taken as the icterus index of the specimen. For example, if the contents of the tube which are three times the strength of the arbitrary unit correspond to the color of the specimen, the icterus index of that specimen is 3. If the color of the specimen is deeper than that of the standards, the specimen is diluted by adding normal saline up to one of the marks on the sample tube. The diluted specimen is compared with the standards and the resulting reading multiplied by the number of times the specimen was diluted. For example, if the specimen is diluted up to the fourth mark on the tube, the reading is multiplied by four. The reading for accurate work may be intergraded between the two tubes which most closely approximate the color of the sample. A check reading may be obtained by reading an undiluted specimen first and then a diluted specimen.

Using this standard, the range of the index for "normal" blood is from 3.5 to 6. A reading below these figures indicates secondary anemia, while a higher figure indicates primary or hemolytic anemia and various degrees and types of obstruction in the biliary system. Clinical jaundice will give an icterus index of 15 or over.

The simplicity of the technic and the accessibility recommends the test, as a set of standards, a reading box and graduated tubes are the only requirements.

PRESERVATION OF BLOOD FOR CHEMICAL ANALYSIS

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That blood on standing undergoes a change in the concentrations of its chemical constituents has long been known. The sugar content of human blood decreases rapidly, as has been shown by Birchard,¹ Denis and Aldrich,² Sander³ and myself (table 1). However, Watson and White⁴ have not been able to demonstrate any material change with time in the sugar content of ox blood. Sander,³ on the other hand, has shown that uric acid, urea and nonprotein nitrogen show an increase in value on standing, although the creatinine and creatine values seldom vary.

Obviously it is of prime importance that analyses give values which correspond to the actual concentrations in the fresh blood, and yet at times it is impossible or impracticable to make the analysis immediately after the withdrawal of the blood. For this reason, several attempts have been made to preserve the blood in some way until a time when analysis is possible. Birchard¹ suggests that the blood be deproteinized immediately after withdrawal, as it keeps well in this state. This is open to the same objections as a complete analysis, as it is nearly as time consuming. Denis and Aldrich² advocate the use of formaldehyde in the ratio of one drop of solution of formaldehyde to 5 cc. of blood, and they give results which seem to show that this is an efficient preservative. Bock,⁵ after the use of a number of different brands of solution of formaldehyde, announces

1. Birchard, D. E.: *J. Lab. & Clin. Med.* **8**:346, 1923.
2. Denis, W., and Aldrich, M.: *J. Biol. Chem.* **44**:203, 1920.
3. Sander, F. V.: *J. Biol. Chem.* **58**:1, 1923.
4. Watson and White: *J. Lab. & Clin. Med.* **1**:1, 1920.
5. Bock, J. C.: *J. Biol. Chem.* **59**:73, 1924.

its use as inadvisable since the results obtained are erratic. Denis and Beven⁶ feel that the use of formaldehyde has many disadvantages as a blood preservative, chief among which is the fact that it cannot be used when urea and nonprotein nitrogen determinations are to be made. Sander⁸ states that the results obtained with the use of formaldehyde vary considerably and are unreliable, especially in blood specimens of high sugar values.

A number of workers have taken up the study of various substances as blood preservatives. Not only have the results been similar, but also sodium or potassium fluoride has been recommended finally in each case, since this seems to be an efficient preservative of all the constituents of the blood that are analyzed clinically and an excellent anticoagulant, so that the use of an oxalate is unnecessary. Aibara⁷ recommends that sodium fluoride be used in a concentration of 0.2 per cent. Denis and Beven⁶ advise that 60 mg. be added to each 10 cc. of blood. Major⁹ suggests that one drop of a saturated solution of potassium fluoride be

TABLE 1.—*Variation in Sugar Content with Time of Oxalated Blood and Blood Preserved with Sodium Fluoride-Thymol*

Patient	Sugar Content, Mg. per 100 Cc.				
	Number of Hours Elapsing Between Withdrawal and Analysis				
	0	3	8	16	24
Oxalated Blood					
W.	150	107	...
B.	250	225	188	...	100
L.	88	...	79
C.	107	...	107
S.	100	...	94
T.	107	...	68
Blood Preserved with Sodium Fluoride-Thymol					
W.	166	166	...
B.	321	321	...
L.	125	...	125
C.	150	...	150
S.	150	...	130
T.	115	...	115

added to each 5 cc. of blood. All of the authors emphasize the necessity of preventing bacterial infection of the collected blood by the use of sterile containers. Sauder⁸ advises that for each cubic centimeter of blood, 0.01 Gm. of sodium fluoride to which has been added 0.001 Gm. of thymol be used. The thymol, being an antiseptic, inhibits bacterial action so that sterile technic is unnecessary except as regards the patient. John⁹ recommends the use of 20 mg. of a 10 to 1 mixture of sodium fluoride and thymol. In the determinations of the authors mentioned in the foregoing, the Folin-Wu system of analysis¹⁰ was used throughout, except that Sander substituted the method of Folin and Denis¹¹ for the determination of uric acid.

6. Denis, W., and Beven, J. L.: *J. Lab. & Clin. Med.* **9**:674, 1924.

7. Aibara, C.: *J. Biochem.* **1**:457, 1922.

8. Major, R. H.: *J. A. M. A.* **81**:1952 (Dec. 8) 1923.

9. John, H. J.: *Arch. Path.* **1**:227 (Feb.) 1926.

10. Folin, O., and Wu, H.: *J. Biol. Chem.* **41**:367, 1920; **38**:81, 1919; **54**:153, 1922.

11. Folin, O., and Denis, W.: *J. Biol. Chem.* **13**:469, 1912-1913.

The long felt need for such a preservative has caused the ready acceptance of sodium fluoride and thymol by many clinical laboratories, and their use is increasing. The study of their action was undertaken by myself, methods of analysis other than the Folin-Wu technic being employed in an attempt to ascertain the effect of this preservative mixture in other analytic systems. Sodium fluoride and thymol were used, as suggested by Sander,⁸ in order that aseptic technic might be eliminated. All analyses were made in accordance with the directions published by Myers.¹² These include the method of Lewis and Benedict¹³ for sugar; the Benedict method¹⁴ for uric acid; Myers' picric acid method for creatinine¹⁵ and Myers' modification¹⁶ of the methods of Marshall,¹⁷ Van Slyke¹⁷ and Folin¹⁸ for urea.

The results obtained for the nitrogenous elements simply corroborate the statements of Sander, John and Denis and Beven that sodium fluoride is an efficient blood preservative, and are, therefore, not given here. The values obtained on blood preserved by the sodium fluoride-thymol mixture for sugar, however, gave decided differences over the oxalated blood when analyzed by the Lewis-Benedict picric acid method instead of the Folin-Wu method heretofore employed. The preservative effect, however, was marked. Table 1 shows the

TABLE 2.—*Differences in Sugar Analysis Values in Oxalated Blood and Blood Preserved by Sodium Fluoride-Thymol from the Same Patient*

Patient	Blood Sugar Values, Mg. per 100 Cc.		
	Oxalated	Sodium Fluoride-Thymol	Difference, per Cent
W.	150	166	10
B.	250	321	28
L.	88	125	42
C.	107	150	40
S.	100	150	50
T.	107	115	7.5

sugar values obtained when the blood of the same patients was preserved by sodium fluoride and thymol, on the one hand, and when it was simply oxalated and allowed to stand at room temperature for varying lengths of time, on the other hand.

A decided decrease in the sugar value occurs in oxalated blood, although the values for blood of these same patients are remarkably constant when the blood has been preserved by sodium fluoride-thymol. It is noted, however, that the sugar value in blood thus preserved is definitely higher than that for oxalated blood. A comparison of the values is given in table 2. In each case the preserved blood gave a greater sugar value, although the percentage difference is not constant, varying from 7.5 to 50 per cent.

12. Myers, V. C.: *Practical Chem. Analysis of Blood*, ed. 2, St. Louis, C. V. Mosby Company, 1924.

13. Benedict, S. R.: *Proc. Soc. Exper. Biol. & Med.* **11**:57, 1913; *J. Biol. Chem.* **20**:61, 1915.

14. Benedict, S. R.: *J. Biol. Chem.* **51**:187, 1922.

15. Myers, V. C.; Fine, M. S., and Lough, W. G.: *Arch. Int. Med.* **17**:570 (April) 1916; *The Post-Graduate*, N. Y. **29**:505, 1914.

16. Marshall, Jr., E. K.: *J. Biol. Chem.* **15**:487, 1913.

17. Van Slyke, D. D., and Cullen, G. E.: *J. Biol. Chem.* **18**:53, 1914.

18. Folin, O., and Denis, W.: *J. Biol. Chem.* **11**:527, 1912.

An attempt was then made to determine the cause of this difference in sugar values in the two methods. A standard solution of dextrose was prepared and treated exactly as though it were blood. Five cubic centimeters were added to the contents of each of five bottles, of which the first contained nothing as a control and the other four contained potassium oxalate, thymol, sodium fluoride and sodium fluoride-thymol mixture, respectively. These preservatives were added in the same proportions as though the standard dextrose solution used had been blood. The solutions were then analyzed in the same manner as blood and their sugar contents determined. Table 3 gives the results. No difference could be obtained through several repetitions. The control solution gave on analysis a sugar value of 160 mg. per hundred cubic centimeters. The oxalated solution also gave this value, showing that oxalate has no effect on the determination. The addition of sodium fluoride to the solution, however, caused a sugar value of 182 mg. per hundred cubic centimeters, while thymol gave a similar rise when added to the solution. Both of these show a rise of 22 mg. per hundred cubic centimeters over the correct value. When sodium fluoride and thymol were used with each other, the sugar value obtained was 208 mg.

TABLE 3.—*Difference in Sugar Analysis Values on Standard Dextrose Solution Mixed with Various Preservatives and Analyzed by the Lewis-Benedict Method*

Blood Sugar Values, Mg. per 100 Ce.		
Preservative	Sugar Content as Analyzed	Increase Over Control
Control.....	160	..
Oxalate.....	160	0
Sodium fluoride.....	182	22
Thymol.....	182	22
Sodium fluoride-thymol.....	208	40

per hundred cubic centimeters. This is an increase of 40 mg. per hundred cubic centimeters over the correct value, or approximately the sum of the increases due to sodium fluoride and thymol alone. It seems, then, that both sodium fluoride and thymol cause, through some chemical participation in the reactions of the analysis, an increase in the value obtained for the sugar content. Each preservative used alone gives the same increase in sugar concentration, and when used with each other, a figure is obtained approximately equal to the sum of the increases for each preservative.

CONCLUSIONS

It has been shown that sodium fluoride and thymol when used in the proportions of 0.01 Gm. of fluoride and 0.001 Gm. of thymol to each cubic centimeter of blood are efficient preservatives when the blood is analyzed by the Folin-Wu system.

This preservative mixture is equally effective for the determination of creatinine, uric acid, urea and nonprotein nitrogen, when the system of analysis as outlined by Victor Myers²² is employed. It may not be used, however, when the sugar values are determined by the Lewis-Benedict method, since consistently greater results varying from 7.5 to 50 per cent are obtained. This increase is apparently due to a chemical participation of the preservatives in the reactions of the analysis.

General Review

THE SUPRARENAL GLAND *

HENRY L. JAFFE, M.D.

NEW YORK

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* From the Laboratory Division of the Hospital for Joint Diseases.

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INTRODUCTION

Within the scope of this general review, not all problems relating to the suprarenals can be discussed. Neither can the entire controversial literature be presented even when some important aspects of this subject are reviewed. My purpose is particularly to emphasize and summar-

ize those advances in the pathology, physiology and pathologic physiology of the suprarenal glands which lead to a clearer understanding of the possible function and relation of these glands to the rest of the organism.

HUMAN ANATOMY

The suprarenal glands are subject to great anatomic variation. The average weight of both glands in normal adults is about 11 or 12 Gm., though in cases of accidental death the reported weight is frequently below 10 Gm.¹ Sex differences vary with age; up to puberty the gland in males is slightly larger; between 16 and 20 years the female suprarenal is larger than the male; after the thirtieth year the suprarenal in the male is again somewhat the larger.² Structurally the gland consists of a folded yellow cortical portion, and a medullary portion which is whitish normally, but reddish brown when congested.

The suprarenals are relatively the most vascular organs in the body, receiving about six times their weight in blood per minute. The blood supply is derived from three vessels which anastomose freely. The capillaries are large and sinusoidal, and pass into veins which eventually unite to form the central vein of the gland, emerging at the hilum. The suprarenal vein contains only longitudinal muscle bundles which follow its ramifications. The thickness of the vein wall varies considerably even in the same vessel, and may in places be reduced to only a few cells.³ The lymphatics are abundant forming a rich network in the medulla which communicates through the cortex with the subcapsular network. The lymph is discharged mainly into lymph glands along the aorta.

The suprarenals are innervated chiefly directly from the splanchnics, also receiving fibers from the suprarenal plexus. These nerves form a network in the connective tissue around the gland. They penetrate it, and run in the connective tissue septa between the cells of the cortex. When the fibers enter the medulla, they come into relation with the sympathetic cells. Some fibers terminate in the cortex. A striking difference exists between the innervation of the cortical tissues and that of the medullary tissues, the latter being richly supplied with nerves, the former sparsely supplied. It has been shown that the activity of the medullary chromaffin tissue is strictly controlled by the nervous system, while the cortex may continue functioning entirely deprived of nerve supply. As to the afferent nervous pathway, nothing definite is known, in spite of much speculation. Elliott⁴ describes a center for the supra-

1. Materna, A.: *Ztschr. f. Konstit.* **9**:1, 1923.

2. Schief, F.: *Ztschr. f. Konstit.* **8**:507, 1922.

3. Kashiwagi, S.: *Tr. Japanese Path. Soc.* **12**:154, 1922. Maresch, R.: *Wien. klin. Wchnschr.* **34**:44, 1921.

4. Elliott, T. R.: *J. Physiol.* **44**:374, 1912.

renals somewhere in the brain stem near the vasomotor centers. Evidently there is a center in the upper thoracic cord, because section of the lower thoracic cord diminishes or abolishes the epinephrine output.

COMPARATIVE ANATOMY

Phylogenetically the suprarenal consists of two distinct organs, but whether they are functionally distinct in either the higher or the lower animals is not known. No organ corresponding to the suprarenal is present in invertebrates, though in certain instances authors have described large cells connected with ganglions which give chromaffin reactions, from which substances giving epinephrine-like reactions have been isolated.⁵ The suprarenal, when it appears in the low vertebrates, consists of cortex and medulla, anatomically separate. In Selachians, for example, the medulla is represented by small paired masses of chromaffin tissue in close association with the sympathetic ganglions, while the cortex, known as the interrenal body, is a large single median structure. The suprarenals of amphibians are structurally intermediate between higher and lower vertebrates. They consist of two paired bodies made up of cells corresponding to those of the interrenal bodies of fishes, with islets of chromaffin cells, irregularly distributed among them, corresponding to the medulla of higher vertebrates. In some reptiles, the chromaffin cells are arranged for the most part on the dorsal aspect of the gland, while in others, there is considerable penetration and mixture of the two elements. In birds the progression toward the mammalian type is more advanced. An intimate interlacement between the cortical and medullary cells exists, so that the latter occupy the meshes of the former. In mammals the chromaffin tissue grows into the cortex during early embryonic life and becomes enclosed by it, and in this way the medulla of the definitive suprarenal is formed.⁶

HISTOLOGY

The cells of the cortex are arranged in three zones descriptively called from without inward: glomerular, fascicular and reticular. In some animals the zones are more clearly defined than in others. On the basis of comparative normal and experimental histologic studies, the glomerular zone is considered the regenerative layer in which the cells continue to multiply throughout life. This conception is supported by the studies of Graham⁷ on the repair of toxic lesions of the suprarenal gland.

7. Graham, G. S.: *J. M. Research* **34**:241, 1916.

Transplantation experiments also confirm this view, the glomerular zone regenerating first.

5. Gaskell, J. F.: *Phil. Tr., Lond., series B* **318**:153, 1914.

6. Vincent, S.: *Internal Secretion and Ductless Glands*, London, Edward Arnold & Co., 1924.

The cortical cells are large, irregular or polyhedral; the nuclei are vesicular; the cytoplasm contains glistening fatlike granules, which blacken with osmic acid, and stain deeply with sudan III and sharlach R. The cells of the reticular zone contain brownish pigment granules—lipochrome.

The cells of the medulla do not show the definite columnar arrangement seen in the cortex, and the cell outlines are not so distinct. The protoplasm of the characteristic cells of the medulla has a great affinity for the nuclear stains, and becomes an emerald green in contact with ferric chloride, as described by Vulpian⁸ in 1856. Henle,⁹ in 1865, discovered that the cells stain any tint between yellow and brown with chrome salts, a reaction used at present for their identification. After the discharge of epinephrine from the chromaffin cells, the color reactions diminish or disappear; hence, they have been attributed to the presence of epinephrine in the cells. The blood of the suprarenal vein, unlike that anywhere else, gives these color reactions because of its relatively high epinephrine content.

EMBRYOLOGY

The suprarenal gland has a dual origin, the cortex being derived from the coelomic epithelium, and the medulla from nervous tissue. The cortex is first recognizable in the human embryo at the beginning of the fourth week (6 mm.), as a series of buds arising from the coelomic cells at the root of the mesentery. Later (from 8 mm. to 12 mm.), these buds become completely separated from the coelomic epithelium, develop an independent blood supply, and form a suprarenal ridge projecting into the coelom between the mesonephros and the root of the mesentery. The central vein becomes viable at about 23 mm. At 19 mm., cells from the adjoining sympathochromaffin tissue migrate toward the cortical mass along its central vein, penetrate it, become completely enclosed by cortex and form the medulla of the gland. The differentiation of the cortex into the three zones does not take place until late in the intra-uterine life. In human fetal suprarenals the presence of epinephrine cannot be detected usually until at term.¹⁰

The comparative embryology of laboratory animals has been worked out carefully, and the development in all species is practically like that in man. In these animals, unlike in man, the chromaffin reaction may appear in the cells of the future suprarenal medulla when small groups of these cells first begin to enter the cortex from the sympathetic ganglion anlage. Inhibition of the beating of the isolated intestinal

8. Vulpian, A.: *Compt. rend. Acad. d. sc.* **43**:223, 1856.

9. Henle, J.: *Ztschr. f. rat. Med.* **24**:143, 1865.

10. Lewis, J. H.: *J. Biol. Chem.* **24**:249, 1916.

strip is obtained with extracts of the suprarenal at this time, indicating that epinephrine or an epinephrine-like substance is already present.¹¹

Formation of Accessories.—As the medullary elements migrate into the cortical anlage in the embryonic development of the suprarenals, fragments of tissue, particularly cortex, are split off with varying frequency in the different species, forming accessories. Only rarely do accessories contain both cortex and medulla; the term is generally applied to cortical rests, which often consist of cells arranged only in glomerular formation; accessory masses of sympathochromaffin tissue are usually not included in this terminology. Most accessories remain near the parent glands, but some become included in or dragged along with such structures as the sex organs, which change their position during development. In this way accessories may be removed from the vicinity of the main glands, appearing in the pelvis or the scrotum.

The Occurrence of Accessories in Man.—Since Morgagni's time accessories have been reported in man in association with practically every organ or structure below the diaphragm. They have measured up to 8 or 9 mm. in diameter, and consisted nearly always of cortex, though some of the larger accessories have also contained medulla. The frequency of occurrence as reported by different authors has varied considerably, because some reported the percentage in the new-born, others in infants and still others in children and adults. According to the data, in the new-born or the very young infant suprarenal accessories can be found in at least 50 per cent of the cases if they are searched for carefully. With advancing age, the accessories undergo atrophy and disappear, because they are physiologically unnecessary in the presence of the main glands. Any figures as to the occurrence of accessories in man, to be acceptable, must be qualified as to the age of the subjects.

The Occurrence of Accessories in Laboratory Animals.—Studies have not been made concerning the frequency and distribution of suprarenal accessories in new-born animals, but from comparative embryology they should be found as often in such animals as in the new-born infant. Wiesel¹² found microscopic accessories in the region of the epididymis in 50 per cent of normal rats at sexual maturity. He obtained evidence of the functional capacities by performing left-sided suprarenalectomies in ten young adult male rats, which he killed from three to twelve weeks later. He found that the cortical accessories in the region of the epididymis had hypertrophied in eight of these animals, some attaining

11. Wiesel, J.: *Zentralbl. f. Physiol.* **2**:614, 1902. Kohn, A.: *Arch. f. mikr. Anat.* **62**:263, 1903. Soulie, A. H.: *J. de l'anat. et de la physiol.* **39**:197, 1903. Weyman, M. F.: *Anat. Record* **24**:299, 1922. Miller, E. H.: *Am. J. Physiol.* **75**:267, 1926.

12. Wiesel, J.: *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. cl.* **108**:257, 1899.

the huge size of 5 mm. Wiesel's work is much quoted, but to my knowledge has never been confirmed. It is doubtful whether after unilateral suprarenalectomy such extreme hypertrophy ever takes place in accessories, because compensation in the animals is rapidly effected by enlargement of the remaining gland. In my experience with several hundred rats of all ages, gross accessories were found in about 8 per cent of normal animals, and in approximately from 20 to 25 per cent after double suprarenalectomy, and they were always situated near the main glands. Careful search for gross accessories in the region of the testis, vas and epididymis always gave negative results in both normal and suprarenalectomized animals. Microscopic accessories were found frequently on serial section of the retroperitoneal tissues from the diaphragm to the pelvis, where they are present in about 70 per cent of rats before sexual maturity.

Gross accessories have been found by Marine¹³ in 70 per cent of rabbits after suprarenalectomy. The difference in size between this animal and the rat probably explains why visible accessories occur so much more often in the rabbit. In the guinea-pig, cat and dog gross accessories are not found frequently, probably because the animals do not survive bilateral suprarenalectomy for a sufficiently long time to permit the microscopic rests to hypertrophy.

INVOLUTIONARY CHANGES IN THE SUPRARENALS AFTER BIRTH

The human suprarenal at birth is about one third as large as the kidney, and the cortex is very thick. This relation changes so that in adult life the kidneys are thirty times heavier than the suprarenals. During the first month of life physiologic degenerative changes take place in the cortex which were independently described in 1911 by Thomas,¹⁴ Elliott and Armour¹⁵ and Kern.¹⁶ Their papers were followed by those of Landau¹⁷ and Lewis and Pappenheimer.¹⁸ The descriptions of these changes by the various observers agree as to the essential lesions. Degeneration is fully established at the end of the second week, and progresses rapidly thereafter, involving the cells of the inner cortical zones, which show extensive nuclear and protoplasmic destruction. The tissue is very vascular, and there is some extravasation of blood. During the next five or six weeks the debris is rapidly removed, leaving an intact thin glomerular layer. The deep cortex is replaced by lamellae of fibrous tissue with cell detritus in the meshes. At 6 months the fibrous replace-

13. Marine, D., and Baumann, E. J.: *J. Metab. Research* **1**:777, 1922.

14. Thomas, E.: *Beitr. z. path. Anat. u. z. allg. Pathol.* **50**:283, 1911.

15. Elliott, T. R., and Armour, R. G.: *J. Path. & Bact.* **15**:481, 1911.

16. Kern, H.: *Deutsche med. Wchnschr.* **37**:971, 1911.

17. Landau, M.: *Die Nebennierenrinde*, Jena, Gustav Fischer, 1915.

18. Lewis, R. W., and Pappenheimer, A. M.: *J. M. Research* **34**:81, 1916.

ment layer appears as a dense connective tissue zone, and the medulla has increased in size. During the succeeding months the connective tissue layer becomes thinner, so that after the first year this fibrous zone remains only where two layers of cortex are adjacent. The thin intact glomerular layer collapses to form the convolutions of the adult cortex. According to Lewis and Pappenheimer, there is not an appreciable increase in the width of the cortex through regeneration up to the age of 3 years, and the birth weight is regained only by the twelfth year. Similar changes have been described in cortical accessory bodies, and the process is considered a systemic one, acting through the blood stream.

These involutionary changes may vary slightly in different persons. Prematurity and inanition do not seem to affect the advent or course, while syphilis may retard them. The changes do not occur in the ordinary laboratory animals.

The cause and significance of this cortical destruction are not known. Marine, Lowe and Cipra,¹⁹ studying heat production in infants, found that the basal metabolism rose during the second week of life, and they associated this with the involution of the suprarenal cortex which begins about the same time. Marine's previous heat production studies in rabbits with sublethal destruction of the suprarenal cortex formed the basis for this conception. These workers theorized that the physiologic destruction was in some way connected with the preparation of the infant for extra-uterine life and growth. They believed that the cortex exerted an inhibitory effect which was diminished by the destruction, causing an activation of many organs.

The degenerative changes in the cortex sometimes extend beyond the normal physiologic limits, leading to massive suprarenal hemorrhage in the new-born, which is pathologic. The hemorrhage may completely destroy the medulla, converting the gland into a blood cyst. Rarely, the cortex ruptures and blood extravasates into the perirenal and retroperitoneal tissues. However, the greater danger is interpreting as pathologic, the physiologic changes present in the suprarenals of infants dying from other causes. Abnormalities in the degree of involution or in the subsequent regeneration may lead to hypoplastic suprarenals, and may be the basis for the suprarenal changes found in status thymico-lymphaticus and for Addison's disease in later life.

PHYSIOLOGY OF THE SUPRARENAL CORTEX EXTIRPATION EXPERIMENTS

In 1856, Brown-Séquard,²⁰ stimulated by the work of Addison and Vulpian, undertook extirpation experiments to ascertain the function of the suprarenals, and announced that these glands were indispensable to

19. Marine, D.; Lowe, B. H., and Cipra, A.: *J. Metab. Research* **2**:329, 1922.

20. Brown-Séquard, C. E.: *Compt. rend. Acad. d. sc.* **43**:422, 1856.

life, because all of his animals, including rabbits and rats, died within a few days after bilateral suprarenalectomy. Though his assertion is correct, the death of his animals so soon after operation was probably due to other factors besides suppression of suprarenal function, since with subsequent improvement of technic, even the most susceptible animals (guinea-pig, dog and cat) survive longer than his animals.

It would not be profitable to review the confused literature of the last seventy years dealing with extirpation experiments. Since more or less uniform results have been obtained recently by reliable observers, the effects of the extirpation of the suprarenal glands in laboratory animals may now be stated generally as follows: No animal can survive bilateral suprarenalectomy for more than a few days in the complete absence of cortical tissue, including accessory rests.

More specifically, the best results thus far reported in the dog are those of Stewart and Rogoff,²¹ who removed the suprarenals in twenty-nine animals in two operations, and found that of twenty-six in which death was not complicated, one died on the second day and two on the third day, while the remaining twenty-three died between the fourth and sixteenth days.

In cats the best reported results are those of Elliott.²² Of twenty-one animals in which both glands were excised at one operation, nineteen died in from eighteen to forty-eight hours. In a series of twenty-five cats in which the glands were removed in two operations, nine died on the second or third day, while thirteen lived from six to ten days, and two survived until the twenty-second and twenty-third days. One cat seemed to have recovered completely and was killed in the ninth week. Elliott believed that the long survival after double suprarenalectomy was due to the hypertrophy of accessory tissue. In a personal communication Marine and Baumann report that their average survival time for 300 cats doubly suprarenalectomized in one operation is five and one-half days.

For guinea-pigs, Rogoff²³ reports that in fifteen uncomplicated cases with an interval of two or three weeks between the removal of the right for 300 cats doubly suprarenalectomized in one operation is five and one-half days.

According to a personal report from Marine, Manley and Baumann, 50 per cent of young adult rabbits survived double suprarenalectomy done in two stages for at least thirty days, and about 15 per cent of these survived normally indefinitely. In the 50 per cent which died during the first month, there were few deaths before the tenth day.

21. Stewart, G. N., and Rogoff, J. M.: *Proc. Soc. Exper. Biol. & Med.* **22**: 394, 1925.

22. Elliott, T. R.: *J. Physiol.* **49**:38, 1914.

23. Rogoff, J. M., quoted by Stewart, G. N.: *Physiol. Rev.* **4**:163, 1924.

Difficulties arose in correlating the results of suprarenalectomy in rats with the idea that the glands were vital to life, for according to reports in the literature this animal withstood ablation of the glands very well. Some authors even reported that removal of the main glands and accessories did not cause death. In Boinet's²⁴ experience with fifty-eight rats doubly suprarenalectomized in one operation, eleven animals, or 19 per cent, were alive several months later, some in good condition. His paper is often misquoted. Rogoff²⁵ reported that fully 50 per cent of rats survive indefinitely the complete removal of both suprarenals. "Indefinitely" should not be used in survival experiments as it does not indicate either the physical condition or the survival period of the animal. Jaffe,²⁶ summarizing his results in ninety young suprarenalectomized rats, which were followed for a year after operation, found that 34 per cent died within thirty days after operation, most of the deaths occurring between the fifth and twelfth days; 46 per cent survived double suprarenalectomy for months, but were chronically insufficient, most of them finally dying from the effects of suprarenal ablation within seven months after operation, and only 19 per cent survived normally in good condition.

The Reason for Survival after Suprarenalectomy.—It is important to tell why some animals die soon after bilateral suprarenalectomy (all dogs, cats and guinea-pigs, and a certain percentage of rats and rabbits), while others live, either for a long time with signs of suprarenal insufficiency, or normally in good condition. The work of many investigators indicates that for survival a suprarenalectomized animal is dependent on the presence of and functional activity of gross or microscopic accessory cortical tissue.

My experience has been confined to the rat and guinea-pig. In the rat suprarenalectomy is never fatal in the presence of active accessory tissue. Perhaps 1 per cent of rats die of suprarenal insufficiency in the presence of gross accessories, but examination shows interstitial fibrosis, vacuolization, distortion of the cells and giant cells, a picture which may be called exhaustion atrophy. In rats dying of acute insufficiency within two weeks after extirpation, gross accessories are not seen. Rats dying of chronic insufficiency months after operation frequently show the presence of exhausted microscopic rests, on examination of the retroperitoneal tissue. To summarize, large active accessories are capable of maintaining suprarenalectomized rats in normal condition, while physiologically active microscopic rests can maintain them, though inadequately, for months. With atrophy and the physiologic exhaustion

24. Boinet, E.: *Compt. rend. Soc. de biol.* **47**:162, 1895.

25. Rogoff, J. M.: *J. Pharmacol. and Exper. Therap.* **26**:243, 1925.

26. Jaffe, H. L.: *Am. J. Physiol.* **78**:453, 1926.

of these accessories suprarenalectomized rats die. If rests are absent or below the threshold quantity, death follows rapidly after suprarenal-ectomy. Similarly, Marine's experience indicates that the survival of suprarenalectomized rabbits is dependent on physiologically active accessories.

Some workers do not agree that accessories maintain suprarenal-ectomized animals, in spite of the evidence in favor of this view, and maintain that for proof accessories must be removed surgically from surviving suprarenalectomized animals. In view of the widespread distribution of accessory tissue, frequently not approachable surgically even when situated near the kidney, the indirect evidence, that is, the presence of large active accessories in animals surviving suprarenal-ectomy, and the exhaustion or absence of accessories in animals that die, should be accepted as proof that cortical accessory tissue maintains suprarenalectomized animals. The occasional presence of gross accessories in cats and guinea-pigs dying after removal of the second gland does not menace this conception. Since the cortex undoubtedly stands in different quantitative relations as to its vital functions in the different species, the accessories in these cases were probably below the vital threshold quantity.

The fact that a suprarenalectomized animal will survive for a few days in apparently good condition, and then suddenly develop serious symptoms of impending death, points out either that the microscopic accessories are capable of maintaining the balance for a few days, or that the mechanism by which the suprarenal normally functions permits wide latitudes immediately after operation. An analogy may be seen in completely thyroidectomized animals, in which a fall in heat production does not occur until about the tenth day after operation.

Acute Symptoms Following Suprarenalectomy.—Excluding those animals in which death is complicated by the shock of operation or hemorrhage, symptoms attributable to the loss of the glands, resulting in death, may develop in animals surviving for either short or long periods after suprarenalectomy. Stewart and Rogoff,²¹ and Banting and Gairns²⁷ have described the acute symptoms following suprarenal extirpation in dogs. According to these authors, the dog usually recovers completely from the removal of the second gland in a few hours. A period of apparently normal health follows, lasting until a few hours before death. The first critical symptom that appears is complete anorexia which comes on rather abruptly, often accompanied or followed by vomiting and diarrhea, but sometimes preceded by them. Blood and bile appear sometimes in both the vomitus and the stool. Weakness is generally not manifested until near death when it is

27. Banting, F. G., and Gairns, S.: *Am. J. Physiol.* **77**:100, 1926.

especially evident in the hind legs if the animal tries to walk or stand. Exaggerated reflex excitability with twitching of the limbs is common, and convulsions with or without coma are occasionally observed. Shortly before death the pulse may be slow and irregular, or rapid. The rectal temperature may be subnormal.

The onset of the serious symptoms and the symptomatology of suprarenalectomy in cats are essentially the same as in dogs.²² In guinea-pigs the onset of the fatal symptoms is acute, loss of appetite and weakness being most prominent, while diarrhea is also seen.

Those rats and rabbits that die from the acute effects of suprarenal suppression also appear to be in good clinical condition until a few hours before the decline, when they refuse food, become lethargic and die, sometimes with convulsions.²⁸

Chronic Symptoms Following Suprarenalectomy.—Marine and Baumann²⁹ have shown that after bilateral suprarenalectomy a syndrome of chronic suprarenal insufficiency may be produced in those rabbits in which a sufficient amount of cortical accessory tissue is present to prevent death, and yet not enough completely to compensate for the loss of both suprarenals. The syndrome of chronic suprarenal insufficiency in the rat, which is easily produced and is clearly recognizable, has recently been described by Jaffe.²⁶ The chief symptoms in both the rat and the rabbit are progressive emaciation, which results in marked or complete absence of fat, asthenia and lowered resistance, which leads in some instances to snuffles and skin infections.

A small percentage of cats, guinea-pigs and dogs may survive bilateral suprarenalectomy for some weeks, but the symptom complex of chronic suprarenal insufficiency cannot be produced in these animals with any degree of regularity. Even if suprarenalectomy is incomplete, the animal may still die as it does after total suprarenalectomy, because the remaining fragment may be too small, or its blood supply may be interfered with. If the fragment left behind is too large the animal may recover completely without any symptoms of suprarenal insufficiency.

Autopsy Observations in Acute Suprarenal Insufficiency.—Death comes abruptly though these animals may have survived suprarenalectomy for as long as two weeks. The observations are essentially the same for all species. They consist mainly of congestion of the internal organs, especially the pancreas and mucous membrane of the duodenum and rectum. The intestines may be filled with a dark brown content. The frequency of congestion, extensive submucosal ecchymosis, hemorrhagic erosions and ulcers of the stomach without inflammatory

28. Marine, D., and Baumann, E. J.: *Am. J. Physiol.* **57**:135, 1921. Jaffe (footnote 26).

29. Marine, D., and Baumann, E. J.: *Am. J. Physiol.* **59**:353, 1922; footnotes 13 and 28 (first reference).

reactions is well-known.³⁰ Lymphoid and thymus enlargement due to regeneration and secondary hyperplasia of the tissues may be seen if the animals have survived for about two weeks, and if emaciation is not too pronounced.³¹ The blood is thick and only small amounts of serum separate out. Pathologic pigmentation of the skin has never been noted. The older observers emphasized changes in the brain and cord, but the importance of these is doubtful.³² In dogs the gum margins are congested and bluish, and sometimes ulcers appear under the tongue. Sections of the liver show degeneration or necrosis of the cords, which may be extensive. There may be focal necrosis of the kidney.²⁷ Gross accessory cortical tissue is rarely found in these animals.

Autopsy Observations in Chronic Suprarenal Insufficiency.—The major observations in such animals (rabbits and rats) are atrophy of the viscera and sex glands, granular kidneys and enlargement of the thymus and lymphoid tissues. The thymic enlargement may be pronounced even in the presence of emaciation. However, most striking is the disappearance of fat which may be complete in animals with insufficiency of long standing. In others there is only a small amount of fat, frequently not more than one-hundredth of the amount present in a normal well nourished animal. In rabbits exhausted gross accessories may be seen, while in the rat a gross accessory is only rarely seen.

Attempts to Prolong the Life of Suprarenalectomized Animals.—Rigid application of certain general principles helps to prolong the survival time of suprarenalectomized animals, and is particularly useful during the early critical period after operation. The animals should be fed carefully, the room temperature should be kept constantly at about 78 F., overcrowding should be avoided and the animals should be tamed so that they do not struggle. Specific therapeutic procedures have been inefficacious. The injection of cortical extracts prepared by many methods, while not giving any encouraging results, has prolonged to some extent the lives of the animals, according to several investigators. The feeding of suprarenal cortex or its extracts has been without results. The injection of cholesterol and its esters, of other lipoids and of epinephrine has been futile in maintaining suprarenal insufficient animals. In evaluating the results of specific treatment, one must consider that palliative but temporary effects have been obtained by some workers merely by the injection of salt solutions to which dextrose has been added. Stewart and Rogoff²¹ injected Ringer's solution and dextrose, intravenously into seven dogs, usually once in twenty-four hours. Of

30. Mann, F. C.: J. Exper. Med. **24**:329, 1916.

31. Jaffe, H. L.: J. Exper. Med. **40**:325, 1924; **40**:619, 1924, and **40**:753, 1924. Marine, D.; Manley, O. T., and Baumann, E. J.: J. Exper. Med. **40**:429, 1924. Crowe, S. J., and Wislocki, G. B.: Bull. Johns Hopkins Hosp. **25**:287, 1914.

32. Tizzoni, G.: Beitr. z. path. Anat. u. z. allg. Pathol. **6**:3, 1889.

the seven dogs, one died on the thirty-third day after removal of the second gland, one on the thirty-fourth day, two on the twenty-first day and one on the sixteenth day. The two remaining animals died on the twelfth and sixth days, but the authors believed that their deaths were complicated by acute pulmonary disease. When the injections are not begun until the animal is moribund, they do not prolong life to nearly the same extent. In a few instances Stewart and Rogoff observed symptoms which were connotative of impending death disappear under the treatment.

Marine,³³ in a large series of cats doubly suprarenalectomized, injected salt solution intraperitoneally and obtained an average survival period of about sixteen days.

RESISTANCE

The Resistance of Suprarenalectomized Animals to Drugs and Toxins.—It was believed that the major function of the suprarenal was to neutralize drugs and toxins. This led to considerable work on the relation of these glands to resistance. The earlier literature is well reviewed in the recent papers of Lewis³⁴ and Scott.³⁵ In rats, between five and thirty days after suprarenalectomy, Lewis reported an increased susceptibility to drugs, especially morphine, the animals being from 400 to 500 times more sensitive than normal rats. Scott also reported marked diminution in the resistance of suprarenalectomized rats to morphine when tested from seven to fourteen days after operation. He found, too, that such animals succumb readily to bacterial intoxication. Jaffe and Marine³⁶ confirmed these results, injecting standard typhoid vaccine intraperitoneally, and showed that in a certain percentage of animals compensation takes place in about nine weeks after suprarenalectomy when the rats withstand doses that would have proved fatal two weeks after operation. Stewart and Rogoff,³⁷ Rogoff and DeNecker,³⁸ and Rogoff and Ecker³⁹ say that if suprarenalectomized rats are tested after having fully recovered from the immediate operative effects, they do not show increased susceptibility to toxins and drugs.

This problem has recently been restudied by Jaffe,⁴⁰ who reports that the removal of both suprarenals in rats, in the absence of accessory

33. Marine, D., personal communication to the author.

34. Lewis, J. T.: *Am. J. Physiol.* **64**:506, 1923.

35. Scott, W. J. M.: *J. Exper. Med.* **38**:543, 1923, and **39**:457, 1924.

36. Jaffe, H. L., and Marine, D.: *J. Infect. Dis.* **35**:334, 1924.

37. Stewart, G. M., and Rogoff, J. M.: *J. Pharmacol. and Exper. Therap.* **19**:97, 1922.

38. Rogoff, J. M., and DeNecker, J.: *J. Pharmacol. and Exper. Therap.* **26**:243, 1925.

39. Rogoff, J. M., and Ecker, E. E.: *Suprarenalectomy and Susceptibility to Tetanus Toxin*, *Arch. Path.* **1**:309 (Feb.) 1926.

40. Jaffe, H. L.: *Am. J. Path.* **2**:421, 1926.

tissue, invariably leads to a marked immediate decrease in the resistance to typhoid vaccine, which is never completely compensated for. That this is a consequence of the loss of some function of the cortex is shown by the fact that hypertrophied accessory rests protect suprarenalectomized rats against otherwise fatal doses of vaccine, and that autoplasmic cortical transplants also protect animals not having any other gross suprarenal tissue.

Anaphylactic Shock and Suprarenalectomy.—Kepinow⁴¹ found that the removal of one suprarenal gland and of the greater part of the other from guinea-pigs makes them more susceptible to anaphylactic shock, especially to active anaphylaxis. Flashman⁴² showed that after suprarenalectomy rats are markedly susceptible to anaphylactic shock produced by intraperitoneal injections of horse serum, though he pointed out, as have others, the difficulty of obtaining true anaphylaxis in this species.

General Resistance Following Suprarenalectomy.—After this operation there is diminished resistance in all species to excessive heat, cold and humidity, to fatigue and to disturbances in diet. Rats and rabbits are more susceptible to natural infections, developing skin diseases, snuffles and snuffles pneumonia much more frequently than their controls.

Physiologic Significance of Diminished Resistance Following Suprarenalectomy.—Because pathologic changes appear regularly in the suprarenals in acute infections and intoxications, it has been held that the suprarenals play an important part in detoxication. This view is further supported by the experimental evidence of diminished resistance after suprarenalectomy, and by the hypertrophy of the cortex in chronic infections. Many think that because of the general character of the diminished resistance following suprarenalectomy and its nonspecific nature, the results are due rather to the effect of suprarenal removal on the rest of the body, than to the loss of a neutralizing mechanism consequent to the removal of the suprarenals.

Antibody Formation after Suprarenalectomy.—Také and Marine⁴³ have reported that rabbits with high grade but sublethal suprarenal insufficiency produce much higher antisheep hemolysin titers than their controls. Jaffe and Marine³⁶ found that suprarenalectomized rats, which were immunized with typhoid vaccine within three weeks following bilateral suprarenalectomy, had agglutinin titers averaging from two to three times greater than their controls. In rats, about six weeks after suprarenalectomy, a difference could not be determined between the

41. Kepinow: Compt. rend. Soc. de biol, **87**:327, 1922.

42. Flashman, D. H.: J. Infect. Dis. **38**:461, 1926.

43. Také, N. M., and Marine, D.: J. Infect. Dis. **33**:217, 1923.

agglutinin response of the normal and that of the suprarenalectomized animals surviving the operation in good condition. The mechanism involved here is not known but it may be related to the question of absorption of toxins following suprarenalectomy.

TRANSPLANTATION

Free Transplantation of the Suprarenal.—The earliest attempts at suprarenal transplantation had mostly negative results.⁴⁴ The first detailed histologic study of successful autoplasmic suprarenal transplantation was made in 1898 by Poll⁴⁵ who reported a large percentage of takes in rats. During the next twenty years a number of investigators, among them the Cristianis,⁴⁶ Hultgren and Andersson,⁴⁷ Schmieden,⁴⁸ Stilling,⁴⁹ Marchand⁵⁰ and Manley and Marine,⁵¹ studied this problem. Some of these authors have, in a certain percentage of their animals, successfully autotransplanted this gland into the kidney, abdominal wall and spleen. The medulla never regenerates in a transplant. Many failures in transplantation have been recorded, especially because the biologic differences between autotransplantation, homotransplantation and heterotransplantation were not fully considered. Often the piece of tissue transplanted was too large. Other authors neglected the fact that the muscle and subcutaneous tissues in some species, particularly the guinea-pig, is extremely sensitive to medullary tissue, as described by Elliott and Tuckett.⁵² In these animals, the epinephrine contained in the transplanted medulla irritates the tissues, often causing necrosis and suppuration of the transplant, or its replacement by fibrous tissue. If cortex only is transplanted, and if it is washed, a large percentage of takes is obtainable even in this animal. The rat offers excellent opportunity for the study of transplants, at least 80 per cent regenerating even when both cortex and medulla are inserted. In this animal regeneration is completed in three or four weeks, after which time the transplant grows and may reach the size of 5 mm., which is as large as a

44. Canalis, P.: Internat. Monatschr. f. Anat. u. Physiol. **4**:312, 1887. Boinet, E.: Compt. rend. Soc. de biol. **2**:162, 1895. Gourfein, M.: Rev. méd. de la Suisse Rom. **15**:67, 1895. Langlois, P.: Physiol. de M. Charles Richet. **4**:17, 1898.

45. Poll, H.: Zentralbl. f. Physiol. **12**:321, 1898.

46. Cristiani, H., and Cristiani, A.: J. de physiol. et de path. gén. **4**:982, 1902.

47. Hultgren, E. O., and Andersson, O. A.: Skandin. Arch. f. Physiol. **9**:73, 1899.

48. Schmieden, V.: Deutsche Ztschr. f. Chir. **70**:453, 1903.

49. Stilling, H.: Beitr. path. Anat. u. allg. Pathol. **37**:480, 1905.

50. Marchand, F.: Deutsche Chir. **16**:1, 1901.

51. Manley, O. T., and Marine, D.: Ductless Glands, J. A. M. A. **67**:260 (July 22) 1916.

52. Elliott, T. R., and Tuckett, I.: J. Physiol. **34**:362, 1906.

normal gland.⁵³ The glomerular zone regenerates first, but as the transplant enlarges all three cortical layers may appear.

Evidence that Free Autotransplants Function.—Though successful free transplantation has been reported frequently, statements that these transplants function are few. The first suggestive reference in the literature was made by Busch⁵⁴ and his co-workers, who reported evidence of functioning transplants in three suprarenalectomized rabbits out of sixty-one, with survival used as an index. They reported finding medullary cells in the successful grafts, which is doubtful. That about 50 per cent of rabbits survive double suprarenalectomy for more than a month in good condition, because of the presence of accessory cortical tissue, must be considered in evaluating Busch's results.

Jaffe and Plavska⁵⁵ obtained evidence of transplant function in a series of experiments in rats and guinea-pigs. In sixty-three rats out of sixty-seven, in which the transplants were large and well vascularized, and in which autopsy showed the absence of accessories, the animals approached the normal litter and sex controls in weight and activity, and withstood doses of vaccine that invariably killed suprarenalectomized rats that had not received transplants.

Zwemer,⁵⁶ after removing both glands in cats and transplanting the cortex into the rectus muscle, reports that the animals lived ten days, whereas the usual survival period was three days. His results are probably explicable on the basis of absorption of the vital cortical substance, rather than on the functioning of the transplants, because experience has shown that suprarenal transplants do not regenerate, and therefore cannot function until at least three or four weeks after insertion.

Jaffe and Plavska's⁵⁵ autoplasmic transplants in guinea-pigs maintained their animals in excellent condition for months after the removal of the second main gland. These results are conclusive of function when compared with the results for nontransplanted suprarenalectomized guinea-pigs, which survive on the average three or four days. The difficulty of transplantation in this animal, as emphasized by Elliott and Tuckett, was diminished or entirely avoided by separating the cortex from the medulla, and dividing the cortex into small fragments, which were transplanted into the rectus muscle after having been washed thoroughly in sterile physiologic sodium chloride solution.

53. Jaffe, H. L., and Plavska, A.: Proc. New York Path. Soc., to be published.

54. Busch, F. C.; Leonard, T. M., and Wright, T.: Further Results in Suprarenal Transplantation, J. A. M. A. **51**:640 (Aug. 22) 1908.

55. Jaffe, H. L., and Plavska, A.: Proc. Soc. Exper. Biol. & Med. **23**:528, 1926. Jaffe (footnote 40). Jaffe and Plavska (footnote 53).

56. Zwemer, R. L.: Anat. Record **29**:103, 1924.

Blodinger, Klebanoff and Laurens⁵⁷ failed to obtain function in autoplasmic and homoplasmic transplants in the dog. In spite of transplantation the animals died soon after the removal of the second gland, and the transplants had degenerated by the thirty-fourth day. Had they transplanted numerous small fragments of cortex only, there would probably have been a fair percentage of takes even in this animal, and functional activity of the transplants might have been demonstrated.

Homotransplantation.—This procedure has been carried out in rabbits by Schmieden⁴⁸ who reported takes in 77 per cent of a series of thirteen animals. Of fifteen rats which we examined one month after homotransplantation four had positive transplants. The experiments were done without considering blood compatibility. It seems even from these few instances, considering the clinical appearance of the animals, that these transplants also may function.⁵⁸

Heterotransplantation.—Thus far heterotransplantation has given negative results in regard to both take and function.

"Stalked" Transplants.—The studies in "Gestielte" transplantation carried out by von Haberer⁵⁹ are actually dislocations of the suprarenal into the kidney. The histologic appearance of his transplants, the finding of medullary tissue, and the statement that these transplants function must be interpreted with the understanding that in these transplantations the original blood and nerve supplies were not severed.

The Clinical Application of Transplantation.—An occasional report appears in the literature on the use of transplantation as a therapeutic measure against Addison's disease. On studying these cases the reasons for the failures become apparent. At operation most of the patients were moribund; in nearly all cases the transplants were heteroplasmic, and generally a whole gland was inserted into some part of the recipient's body. If this question were carefully reworked, utilizing the facts now known about suprarenal transplantation, a certain small percentage of patients with Addison's disease would obtain some therapeutic relief from this treatment, which would at least be palliative.

METABOLISM

Relation of the Suprarenals to Heat Production.—Marine and Baumann²⁹ studied heat production in rabbits suffering from sublethal suprarenal insufficiency induced by bilateral extirpation of the glands or by freezing the cortex. In rabbits with chronic suprarenal insufficiency, metabolism is measurably increased for three weeks or more,

57. Blodinger, I.; Klebanoff, H. E., and Laurens, H.: *Am. J. Physiol.* **76**:151, 1926.

58. Jaffe, H. L., unpublished data.

59. Von Haberer, H.: *Arch. f. klin. Chir.* **86**:399, 1908, and **94**:606, 1911.

with or without slight remissions, and then gradually falls until death. In animals surviving indefinitely they found that the metabolism, after a rise, may drop a little below the control rate. Increased heat production did not occur when both the thyroid and suprarenals were removed. From their observations they concluded that the increased heat production following sublethal suprarenal insufficiency resulted from a disturbance of an interrenal (cortex) = thyroid relationship. In rabbits dying within a week after removal of the second suprarenal, the result was a steady fall in the metabolism, corresponding to what Aub, Forman and Bright⁶⁰ observed in cats. Marine and Baumann believe that Aub's result—that is, a progressive fall in metabolism after suprarenalectomy—is due to the fact that cats are generally moribund from the time the second gland is removed, and therefore that he was measuring the metabolism in dying animals. According to Scott,⁶¹ severe but non-fatal injury to the suprarenal cortex by freezing or by ligation of the suprarenal veins caused, even in the cat, a significant and prolonged increase in heat production. Lethal injury to the suprarenals caused a progressive fall in heat production corresponding to that obtained by Aub. When their results are correlated, real discrepancy does not exist between the Aub and Marine groups.

The significance of these results is not understood. The increased heat production following sublethal cortical injury seems to be related to a disturbance in the metabolism incident to compensation. The metabolic observations do not explain the marked progressive loss of fat, emaciation and cachexia, from which the rabbit and rat suffer if they die from insufficiency months after suprarenalectomy—nor do they explain the marked metabolic disturbance resulting in death, which follows when cortical removal is more complete.

The Relation of Cholesterol to Suprarenal Physiology.—Since lipoids sometimes constitute as much as 15 or 20 per cent of the fresh weight of the suprarenal cortex many investigations have been made to determine the relation of the lipoids to suprarenal physiology. Grigaut⁶² studied the cholesterol content in the blood of the dog, following unilateral suprarenalectomy, and Rothschild⁶³ studied the cholesterol content in the blood of the rabbit after unilateral and bilateral suprarenalectomy. After unilateral extirpation both reported a gradual increase in the cholesterol content of the blood, which fell to normal within three weeks after operation. After bilateral extirpation, Rothschild reported a continuing rise in the cholesterol content until the animals succumbed, usually within twenty-four hours. From

60. Aub, C.; Forman, J., and Bright, E. M.: *Am. J. Physiol.* **61**:326, 1922.

61. Scott, W. J. M.: *J. Exper. Med.* **36**:199, 1922.

62. Grigaut, A.: *Thèse de Paris*, 1913.

63. Rothschild, M. A.: *Beitr. z. path. Anat. u. z. allg. Pathol.* **60**:39, 1915.

the observations of Grigaut, Chauffard and his school⁶⁴ concluded that cholesterol is a hormone produced by the suprarenal cortex, and that the hypercholesteremia following unilateral suprarenalectomy is the result of compensatory hypersecretion of the remaining gland. The Aschoff school advanced the theory, based on the work of Rothschild and of Landau and McNee,⁶⁵ that the suprarenal is a storehouse for cholesterol which, therefore, accumulates in the body after suprarenalectomy.

Baumann and Holly⁶⁶ recently restudied this question using rabbits, and concluded that the suprarenals are not the seat of cholesterol formation or the storehouse for this substance. They found no significant change in the cholesterol content of the blood of suprarenalectomized rabbits until one week before death, when it rose. They explained the terminal cholesterinemia as a premortal phenomenon. They could not establish any relationship between the plane of metabolism and the blood lipoids, as was recently advocated by Epstein and Lande.⁶⁷

This view of Baumann and Holly has been confirmed recently by Randles and Knudson⁶⁸ and by Lucas,⁶⁹ and is accepted among physiologists and biologic chemists in this country.

Other Blood Chemistry Observations after Suprarenalectomy.—No specific changes in the blood constituents, including lipid phosphorus, have been noted following suprarenalectomy, which may be attributed with certainty to this procedure. Variations from the normal are due to premortal changes and particularly to changes in the concentration of the blood.

Feeding of Suprarenal Glands to Normal Animals.—R. G. and A. D. Hoskins⁷⁰ fed as much desiccated suprarenal as could be given without causing digestive disturbances to a series of 20 day old rats for a period of from two to eight weeks. As a result, the ovaries and testes were larger in those animals fed suprarenal than in the controls, and they concluded that the effect on the gonads must be due largely to the cortical portion of the gland, because very little of the epinephrine con-

64. Chauffard, A.; Laroche, G., and Grigaut, A.: *Compt. rend. Soc. de biol.* **70**:20, 70, and 108 and 536, 1911, and **73**:23, 1912. Chauffard, A.; Richet, C., Jr., and Grigaut, A.: *Compt. rend. Soc. de biol.* **70**:276 and 317, 1911.

65. Landau, M., and McNee, J. W.: *Beitr. z. path. Anat. u. z. allg. Pathol.* **58**:667, 1914.

66. Baumann, E. J., and Holly, O. M.: *J. Biol. Chem.* **55**:457, 1923.

67. Epstein, A. A., and Lande, H.: *Studies on Blood Lipoids*, *Arch. Int. Med.* **30**:563 (Nov.) 1922.

68. Randles, F. S., and Knudson, A.: *J. Biol. Chem.* **67**:17, 1926.

69. Lucas, G. H. W.: *Am. J. Physiol.* **77**:114, 1926.

70. Hoskins, R. G., and Hoskins, A. D.: *Suprarenal Feeding*, *Arch. Int. Med.* **17**:584 (April) 1916.

tained in the medulla is absorbed through the alimentary canal. McKinley and Fisher⁷¹ repeated this experiment on white rats between the ages of 14 and 21 days. After eleven weeks, rats fed on fresh cortex weighed 10 per cent more than their controls, and the testes were 21 per cent heavier. The feeding of fresh whole ox suprarenal gland proved toxic in 0.5 Gm. quantities, because of the medulla.

Marine, Baumann and Cipra⁷² fed glycerol emulsions of the suprarenal cortex to eighteen rabbits. The period of feeding varied from eleven to nineteen days, and the amount administered was from 4 to 10 cc. daily. In eleven animals they observed a fall in the metabolism, varying from 6 to 27 per cent, which usually began about a week after feeding and persisted in most cases for a week after feeding ceased.

Cholesterol Feeding.—When cholesterol is fed to animals, the store of this substance is rapidly increased, and concomitantly the suprarenals enlarge.⁷³ McMeans⁷⁴ interprets this as part of a compensatory mechanism on the part of the body to rid the blood of an excess of cholesterolin, but this is doubtful. It seems, rather, that the suprarenal cortical cells have a great affinity for lipoids.

HYPERTROPHY OF THE SUPRARENAL CORTEX

Hypertrophy of the suprarenal cortex occurs under a wide variety of experimental and physiologic conditions. The condition as it occurs during pregnancy and lactation in man and animals is well known.⁷⁵ Riddle⁷⁶ recently reported hypertrophy during ovulation in pigeons, and Watrin⁷⁷ observed it during the estrum of lower animals. J. C. Donaldson⁷⁸ did not find hypertrophied suprarenals in pregnant rats. The physiologic significance and importance of these observations is not known, since both in animals and in man hypertrophy of other organs and glands also takes place. The understanding of this reaction is further complicated because enlargement of the suprarenal cortex has been reported after gonadectomy in the rabbit, guinea-pig and dog.⁷⁹

71. McKinley, E. B., and Fisher, N. F.: *Am. J. Physiol.* **76**:268, 1926.

72. Marine, D.; Baumann, E. J., and Cipra, A.: *Am. J. Physiol.* **72**:248, 1925.

73. Bailey, C. H.: *J. Exper. Med.* **23**:69, 1916.

74. McMeans, J. W.: *J. Med. Research* **33**:481, 1915.

75. Guieysse, M. A.: *Compt. rend. Soc. de biol.* **51**:898, 1899. Kolmer, W.: *Zentralbl. f. Physiol.* **25**:1009, 1911-1912. Kolde, W.: *Arch. f. Gynäk.* **99**:272, 1913. Schenk, F.: *Beitr. z. klin. Chir.* **67**:316, 1910. Verdozzi, C.: *Arch. ital. de biol.* **66**:121, 1917.

76. Riddle, O.: *Proc. Soc. Exper. Biol. & Med.* **19**:280, 1922.

77. Watrin, J.: *Rev. méd. de l'est.* **48**:349, 1920.

78. Donaldson, J. C.: *Am. J. Physiol.* **68**:517, 1924.

79. Theodossieff, H. E.: *Russk. Vrach.* **5**:135, 1906. Kolmer (footnote 75, second reference). Donaldson (footnote 78).

The suprarenals enlarge during inanition.⁸⁰ McCarrison⁸¹ noted suprarenal enlargement in guinea-pigs on a scorbutic diet. Thyroid feeding causes suprarenal enlargement,⁸² but the interpretation of this observation is not clear, since hypertrophy occurs in other organs, and the suprarenal is said to hypertrophy also after thyroidectomy.⁸³

The hypertrophy of the suprarenal cortex in chronic infections and intoxications is well known. Recently, Mulon and Porak⁸⁴ found cortical hypertrophy in animals used to produce various antibodies. The largest suprarenals were found in rabbits repeatedly injected with sheep's erythrocytes for amboceptor production.

Compensatory Hypertrophy.—Rapid hypertrophy of the remaining gland follows unilateral suprarenalectomy and may involve the accessory cortical rests. This hypertrophy reaches its height within two or three weeks. The consensus of opinion is that the hypertrophy is confined to the cortex. McKay and McKay⁸⁵ report that the compensatory hypertrophy in the rat amounts to 61 per cent fourteen days after unilateral suprarenalectomy.

CHEMISTRY OF THE SUPRARENAL CORTEX

Considerable work has been done on the chemistry of the cortex, particularly with the idea of isolating an active principle and finding the relation of cortical substances to the production of epinephrine, but it has as yet yielded little information. Of the chemical constituents of the cortex there is little knowledge. Analyses have shown the presence of cholin, neurin, stearic and palmitic acids, and sphingomyelin and phrenosin. Specific proteins have not been isolated. Little is known concerning the nature of the anisotropic substances and their relation to the secretion.

The fundamental knowledge of the suprarenal gland was advanced when Oliver and Schaefer,⁸⁶ in 1894, discovered that extracts of the medulla, when injected into the circulation in great dilutions, caused a remarkable but brief rise in blood pressure, mainly as a result of general

80. Stewart, C. A.: J. Exper. Med. **25**:301, 1918. Jackson, C. M.: Am. J. Anat. **25**:221, 1919.

81. McCarrison, R.: Brit. M. J. **2**:200, 1919.

82. Hoskins, E. R.: J. Exper. Zool. **21**:295, 1916. Hoskins, R. G.: Am. J. Physiol. **26**:426, 1910.

83. Kojima, M.: Quart. J. Exper. Med. **11**:255, 1917. Gley, A.: Arch. internat. de physiol. **14**:175, 1914. Carlson, A. J.: Discussion, J. A. M. A. **67**:1484 (Nov. 18) 1916. Stewart, G. N., and Rogoff, J. M.: Am. J. Physiol. **56**:220, 1921.

84. Mulon, P., and Porak, R.: Compt. rend. Soc. de biol. **77**:273, 1914.

85. McKay, E. M., and McKay, L. L.: J. Exper. Med. **43**:395, 1926.

86. Oliver, G., and Schaefer, E. A.: J. Physiol. **16**:1, 1894; **17**:9, 1895, and **18**:230, 1895.

arteriolar constriction. Independently of them, Cybulski⁸⁷ obtained the same results, and he further demonstrated this pressor substance in the blood of the suprarenal vein. Intensive investigations directed toward the isolation of the pressor principle followed and were begun in this country by Abel.⁸⁸ He isolated the suprarenal principle as a monobenzoyl product. Several years later Takamine⁸⁹ and Aldrich⁹⁰ isolated it as a free base. The trade name adopted by Takamine and Aldrich was adrenalin, while Abel's name, epinephrine, has been the name accepted by the Council on Pharmacy and Chemistry. In the U. S. Pharmacopeia this name is also employed, the solution being described as epinephrine hydrochloride. The two suprarenals of an adult contain at most only 9 mg. of the principle. After acute infections and various exhausting diseases, the epinephrine content is much reduced, but on recovery regeneration of the substance occurs within a few days. Hartman⁹¹ believes that the cortex shares in the formation and discharge of epinephrine, but this is not accepted generally.

Epinephrine oxidizes readily on exposure to the air, it turns pink, then brown and finally yields a dark amorphous substance of the melanin group. It is also responsible for the dark color which develops in the medullary portion of the gland after exposure to the air for some time. This chemical reaction is applied clinically to bring out latent pigmentation in Addison's disease by exposure to sunlight in suspected cases. The Vulpian ferric chloride color reaction is produced by the epinephrine, and while not entirely proved, apparently the chrome reactions of the medulla are also entirely dependent on this substance.

Pharmacology of Epinephrine.—Experimentation has shown that epinephrine stimulates or inhibits the action of practically all structures innervated by the sympathetic nerves going to the part. For example, it accelerates the heart rate in the same way as electrical stimulation of the accelerator or sympathetic nerves to the heart. Like stimulation of the splanchnic, it relaxes the stomach and the intestines, but causes increased tonus of the pyloric, the ileocecal and the internal anal sphincters. The injection of solutions of epinephrine causes the following conditions: an increased secretion of saliva, lachrymal fluid, bile and sweat (this last only in horses and sheep; contraction of the erectors pilorum, the gravid and nongravid uterus in many animals and the trigone of the bladder; relaxation of the nongravid uterus in the cat, rat, mouse and guinea-pig; relaxation of the fundus of the bladder, of the coronary vessels and the bronchi (the last, particularly, if the ani-

87. Cybulski, N.: *Zentralbl. f. Physiol.* **9**:172, 1895.

88. Abel, J. J.: *Bull. Johns Hopkins Hosp.* **12**:80, 1901.

89. Takamine, J.: *Am. J. Pharm.* **73**:523, 1901.

90. Aldrich, T. B.: *Am. J. Physiol.* **5**:457, 1901.

91. Hartman, F. A., and Hartman, W. E.: *Am. J. Physiol.* **65**:623, 1923

mal has previously been in a state of spasm); dilatation of the pupil and exophthalmos; polycythemia, and also the conversion of the tissue glycogen into blood sugar. The effect of epinephrine in great dilutions is different from that in more concentrated solutions. Epinephrine is not a vasoconstrictor or a vasodilator, but is dependent on the dosage employed, the organ in which the action is taking place, and the general condition of the organism. It is now accepted that in great dilutions epinephrine produces a pure depressor or dilatation effect.

The point of action of epinephrine is on an intermediate anatomic unit, the myoneural or adenoneural junction. Epinephrine is of considerable importance in practical medicine. Its extraordinary ability to cause constriction of small arteries and veins whenever applied to them, its power to cause relaxation of spastically contracted bronchioles and its power to resuscitate an arrested heart are among the reasons for its widespread use.

Epinephrine in the Suprarenal Vein Blood.—Naturally, when a drug exhibits such pharmacologic potency on injection into the body, considerable interest and discussion is raised as to its action when it is liberated from the suprarenal medulla. Attempts have been made to measure its rate of liberation and concentration in the blood. By the rabbit intestine segment method Stewart and Rogoff⁹² estimated that the average output of epinephrine per minute per kilogram of body weight in 103 cats was 0.000226 ± 0.000007 mg., and for thirty-two dogs 0.000227 ± 0.0000096 mg. In nearly 85 per cent of the cases the output lay between 0.00010 and 0.00030 mg. Their assays show that the concentration of epinephrine in the suprarenal vein of etherized cats is between 1:1,300,000 and 1:9,000,000, and in dogs between 1:1,800,000 and 1:18,000,000. They continually stress the point—that there is a great difference between output and concentration, explaining that concentration varies inversely with the rate of blood flow through the suprarenals while the output is constant. Hoskins and McClure,⁹³ also using the rabbit intestine segment method, estimated that the concentration of epinephrine in the suprarenal vein of dogs was between 1:2,000,000 and 1:8,000,000 of a solution of epinephrine hydrochloride. Assuming that such a solution contains about 70 per cent of the base, their figures agree very well with those of Stewart and Rogoff.⁹⁴ Many other investigators, using different methods, reported on both the output and the concentration of epinephrine. The figures vary; some are twenty times greater than those given above. Because

92. Stewart, G. N., and Rogoff, J. M.: *Am. J. Physiol.* **66**:235, 1923.

93. Hoskins, R. G., and McClure, C. W.: *The Adrenal Glands and Blood Pressure*, *Arch. Int. Med.* **10**:343 (Oct.) 1912.

94. Stewart, G. N.: *Physiol. Rev.* **4**:163, 1924.

of the inherent difficulties in these estimations, discrepancies in the assays will appear, and the results of inexperienced workers must be interpreted cautiously.

Destruction of Epinephrine in the Body.—Epinephrine has never been detected with certainty in the peripheral blood of man or animal in health or disease. Its concentration in arterial blood has been estimated as 1:1,000,000,000. What becomes of the epinephrine present in the suprarenal vein, and how it is destroyed in the body, are not exactly known. That it is not excreted as such is generally believed. It has not been recognized in any of the excretory fluids of man, though it has been found in the dermal secretion of a West Indian toad.⁹⁵ When epinephrine is infused into arteries, and its concentration in the arterial blood is raised much above the physiologic level, most of it disappears in passing through the tissue capillaries. It has been shown further that most of it disappears in passing through the tissues or organs in which it induces a reaction. This explains why it cannot be detected in the peripheral venous blood.

Nervous Control of Epinephrine Secretion.—Dreyer⁹⁶ showed that stimulation of the peripheral end of the splanchnic nerve causes an increase in the amount of epinephrine entering the blood of the suprarenal vein. This work was confirmed and extended by Tschoboksaroff,⁹⁷ Asher,⁹⁸ Joseph and Meltzer,⁹⁹ and Elliott.⁴ That the spontaneous output of epinephrine is entirely under the control of nerves is also well established. Stewart and Rogoff¹⁰⁰ have shown that the epinephrine output in the cat or dog was either greatly diminished or nil after section of the nerves to the suprarenal. Most of the efferent epinephrine secretory fibers run in the major and minor splanchnic nerves; and there is evidence that some fibers may pass from the abdominal sympathetic chain to the glands.

Little is definitely known of the part played by the central nervous mechanism in controlling the secretion, but it seems that the uppermost thoracic segments are related to the epinephrine output. By section of the lower thoracic cord, this center of control is removed, and the epinephrine output is diminished or abolished. Definite evidence as to centers situated in the brain is not found, though from the present knowledge of the cerebral control of the spinal centers, some such mechanism may exist.

95. Abel, J. J., and Macht, D. I.: *J. Pharmacol. & Exper. Therap.* **3**:319, 1911.

96. Dreyer, G. P.: *Am. J. Physiol.* **2**:203, 1899.

97. Tschoboksaroff, M.: *Arch. f. d. ges. Physiol.* **137**:59, 1911.

98. Asher, L.: *Ztschr. f. Biol.* **58**:274, 1912.

99. Joseph, D. R., and Meltzer, S. J.: *Am. J. Physiol.* **29**:34, 1911-1912.

100. Stewart, G. N., and Rogoff, J. M.: *J. Pharmacol. & Exper. Therap.* **10**:1, 1917.

Concerning the afferent paths through which the epinephrine output can be affected, there has been considerable controversy, and two schools of opinion have developed. This controversy has centered around the theory of emergency function, propounded by Cannon¹⁰¹ in 1914.

THE THEORY OF THE EMERGENCY FUNCTION OF THE SUPRARENALS

Cannon and de la Paz,¹⁰² and Cannon and Hoskins¹⁰³ in 1911 reported that the suprarenal medulla was stimulated to secrete during emotional stress, pain and asphyxia. Cannon and Nice¹⁰⁴ and Gruber¹⁰⁵ then pointed out that epinephrine lessened muscular fatigue, while Cannon and Mendenhall¹⁰⁶ showed that it accelerated the coagulation of blood. The results of these and numerous other studies by the Cannon group were elaborated into the emergency function theory of the suprarenals, which introduced a new conception of the rôle of the sympathetic system of the suprarenal medulla in important bodily adjustments.

The emergency theory was presented by Cannon and his co-workers on the basis of studies of suprarenal secretion following the stimulation of afferent sensory nerves, asphyxia and emotional excitement. In their papers bearing on this subject they emphasize that the secreted epinephrine under these circumstances did not have a different function separate from that of nerve impulses, and that the changes in the viscera induced by nervous impulses were continued only by the circulating epinephrine. They also state that during serene existence epinephrine is unnecessary and is, in fact, not secreted; they believe that it enters the circulation only in times of great emotional stress and under circumstances causing pain and asphyxia, when peristalsis is inhibited and blood is shifted to the vital organs as the lungs, heart, central nervous system and skeletal muscles. The effects of fatigue are overcome quickly, and sugar is mobilized into the circulation. Under such conditions epinephrine aids the sympathetic division of the autonomic system to render the organism most efficient for combat, flight or pursuit.

The evidence on which this theory was based and its interpretation have been questioned particularly by Stewart and Rogoff. On the basis of apparently careful quantitative studies, they believe that the discharge of epinephrine is continuous, that the amount is approximately constant per unit of time and that a more or less constant output exists in the ordinary species of laboratory animal, reckoned in proportion to

101. Cannon, W. B.: *Am. J. Physiol.* **33**:356, 1914.

102. Cannon, W. B., and de la Paz, D.: *Am. J. Physiol.* **28**:64, 1911.

103. Cannon, W. B., and Hoskins, R. G.: *Am. J. Physiol.* **29**:274, 1911-1912.

104. Cannon, W. B., and Nice, L. B.: *Am. J. Physiol.* **32**:44, 1913.

105. Gruber, C. M.: *Endocrinology* **3**:145, 1919.

106. Cannon, W. B., and Mendenhall, W. L.: *Am. J. Physiol.* **34**:243, 1914.

body weight. Stewart and Rogoff were unable to find an increased output of epinephrine in pain, asphyxia or emotional excitement, believing that the increase obtained by other observers under these conditions was an increase not in output, but merely in concentration, due to a diminution in the rate of blood flow through the lumbosuprarenal veins.¹⁰⁷ According to them, different anesthetics, operative procedures and the various methods of collecting blood from the suprarenal vein do not have an appreciable effect on the output of epinephrine. To review the work of other investigators, who support or oppose either of these views, would not give much further satisfaction.

Thus two schools working on the same problem have reached antipodal conclusions. The controversies have been centered chiefly about the value of the methods used. The validity of the denervated or isolated heart,¹⁰⁸ as an indicator of increased epinephrine secretion, has been most strenuously objected to by Stewart and Rogoff.¹⁰⁹ It seems that Cannon has himself given a satisfactory answer and has brought forward evidence by other workers to refute the objections to the method.¹¹⁰ The assumption of Stewart and Rogoff that the results obtained by Cannon are due to shifts of the circulation, with consequent increased concentration of epinephrine in the suprarenal or caval blood, needs further proof.

Cannon's contention that epinephrine is not secreted during serene or peaceful conditions seems untenable because the medulla is markedly vascular and is controlled by an abundant nerve supply which increases the epinephrine output on electric stimulation, and which leads, when sectioned, to a diminution or abolition of the epinephrine store and output. Furthermore, whenever suprarenal vein blood has been tested, it has been found to contain epinephrine. It is hardly conceivable, therefore, that the medulla should not have a constant secretion, as Stewart and Rogoff have claimed. This constant secretion may be regarded as the "vegetative" level, which should become augmented, and there is no logical reason why it should not, when physiologic nervous stimuli reach the suprarenal medulla through the sympathetic system during periods of emotional stress. That stimuli reach other abdominal organs through the autonomies during periods of stress is well known. However, whether the increased epinephrine output that

107. Stewart, G. N., and Rogoff, J. M.: *J. Pharmacol. and Exper. Therap.* **8**:517, 1916, and **10**:49, 1917; *Am. J. Physiol.* **52**:528, 1920; *J. Exper. Med.* **26**:637, 1917; *J. Pharmacol. & Exper. Therap.* **8**:517, 1916; *Am. J. Physiol.* **52**:304, 1920.

108. Cannon, W. B.: *Am. J. Physiol.* **50**:339, 1919.

109. Stewart, G. N., and Rogoff, J. M.: *Am. J. Physiol.* **46**:90, 1918.

110. Cannon, W. B., in Barker, L. F.: *Endocrinology and Metabolism*, New York, D. Appleton & Co., 1922, vol. 2.

occurs during these periods plays as important a rôle as Cannon believes is not known.

FUNCTION OF EPINEPHRINE IN BODILY PROCESSES

Whatever these functions may be, the abrupt suppression of the epinephrine output from the suprarenals does not cause any obvious changes. The part played by the extramedullary chromaffin tissue in the liberation of epinephrine and in compensation for the suppression of epinephrine from the main glands is questionable. After the suppression of the epinephrine output in the dog, an immediate fall in the blood pressure attributable to this procedure does not occur.¹¹¹ Henderson, Prince and Haggard¹¹² have shown that during the period of low blood pressure incident to shock, attempt at compensation by an increase in the epinephrine output does not take place.

Since Elliott's time¹¹³ some have believed that epinephrine secreted under physiologic conditions activates or increases the irritability of the sympathetic system or the myoneural junction, the intermediate link between the nerve ending and the muscular fibers. This gave rise to the theory that the function of epinephrine was to increase tonus. Cannon does not accept the tonus theory, believing that epinephrine functions only during times of stress, when it augments the effect of the autonomic nervous system. Under pharmacologic conditions epinephrine undoubtedly acts on the myoneural junction.

The effects of epinephrine in physiologic amounts on normal metabolism are not known. To what degree chronic suprarenal insufficiency is dependent on epinephrine insufficiency, I cannot say, but animal experiments indicate that its deficiency does not produce this condition. Most of the information concerning the influence of epinephrine on metabolism is based on the results of the injection of amounts much greater than any occurring physiologically. Basal metabolism and rectal temperature are unaffected by operations which suppress the normal epinephrine output. Morphine, which causes marked hyperthermia in normal cats, produces the same effect on cats in which the epinephrine output has been interfered with.

The influence of epinephrine on sugar metabolism has been a subject of discussion since Blum¹¹⁴ found that the injection of epinephrine caused a glycosuria. Because of this it was believed that the increased blood sugar in experimental hyperglycemias was due to an increased

111. Gley, E., and Quinquaud, A.: *J. de physiol. et de path. gén.* **19**:356, 1921.

112. Henderson, Y.; Prince, A. L., and Haggard, H. W.: *Shock*, *J. A. M. A.* **69**:965 (Sept. 22) 1917.

113. Elliott, T. R.: *J. Physiol.* **32**:401, 1905.

114. Blum, F.: *Arch. f. d. ges. Physiol.* **90**:617, 1902.

liberation of epinephrine from the suprarenals. Carrasco-Formiguera ¹¹⁵ found that piqûre caused a temporary increase in the epinephrine output, but he did not believe that the piqûre hyperglycemia was due to the increased epinephrine output. It has been shown further that piqûre hyperglycemia may take place in the absence of the suprarenals. The hyperglycemia of ether, asphyxia and emotion is believed by some to be due to an increased epinephrine discharge during these states. Stewart and Rogoff doubt whether a true emotional hyperglycemia exists, and do not believe that the hyperglycemias of asphyxia and ether are due to an increased epinephrine discharge.

Keeton and Ross ¹¹⁶ and Kahn ¹¹⁷ believe that epinephrine stimulates glycogenolysis and may assist glycogenolysis produced by other means. Stewart and Rogoff contend that there is no evidence that the rate at which glycogen is transformed into dextrose, or the rate at which dextrose is oxidized in the organism, is influenced by the epinephrine liberated from the suprarenal. The theory that epinephrine is a physiologic antagonist of the pancreatic hormone, and that when the pancreas is removed diabetes follows because of the unchecked secretion of epinephrine, is also unfounded, according to these authors.

From what has been said it is evident that if the emergency function theory of epinephrine is excluded, little is known of how epinephrine acts in the body under normal physiologic conditions.

THE RELATION BETWEEN CHROMAFFIN TISSUE AND THE THYROID

Epinephrine injected intravenously causes a marked constriction of the thyroid vessels. Asher and his co-workers ¹¹⁸ have shown that the blood pressure response to a given dose of epinephrine in rabbits in which the thyroid nerves were stimulated was greater than the response obtained without stimulation of these nerves. The Goetsch epinephrine test in exophthalmic goiter is a clinical application of this reaction. Asher and Flack believe, but this is not proved, that the thyroid hormone increases the irritability of, or sensitizes the tissues innervated by, the sympathetic nervous system, making them more susceptible to epinephrine. The work of Marine and Baumann ²⁹ and of Scott ⁶¹ on heat production, which has been discussed, points to a relationship between the suprarenal cortex and the thyroid.

INDISPENSABILITY OF THE MEDULLA AND EPINEPHRINE TO LIFE

It has already been pointed out that the suprarenal cortex is vital to life. In regard to the medulla, the available evidence indicates that it is not indispensable to life and health. Cats, dogs and monkeys have

115. Carrasco-Formiguera, R.: *Am. J. Physiol.* **61**:254, 1922.

116. Keeton, R. W., and Ross, E. L.: *Am. J. Physiol.* **48**:146, 1919.

117. Kahn, R. H.: *Arch. f. d. ges. Physiol.* **168**:326, 1917.

118. Asher, L., and von Rodt, W. E.: *Zentralbl. f. Physiol.* **26**:223, 1912.

been known to live indefinitely in good health after excision of one suprarenal and section of the nerves to the other, an operation which either abolishes the output of epinephrine or reduces it to a small fraction of the normal. What part the epinephrine of the extracapsular chromaffin tissue plays in maintaining a decapsulated animal is not definitely known. Some workers believe that a complete cessation of epinephrine output can never be obtained on account of these tissues, but their secretion must be insignificant compared to the amount secreted by the medulla of the main glands. A strong argument against the view that the extrachromaffin tissue exerts any influence on life was made by Vincent,¹¹⁹ who pointed out that from the relatively large size of the "abdominal chromophil body" in the dog, this animal might be expected to be quite resistant to suprarenal extirpation, while the white rat, which is quite resistant to this operation, has no extra-suprarenal "chromophil" tissue. For the present, the view must be accepted that the medulla and its secretion are not indispensable to life.

PATHOLOGY, ANATOMY AND PHYSIOLOGY VARIOUS CONDITIONS

Complete Absence of the Suprarenal Tissue.—This has been reported in rare cases after birth, but the reports were probably the result of faulty observation. Either the main glands were displaced or large accessory rests were present but not found.

Aplasia or Hypoplasia.—Nonviable monsters, especially those with extensive cerebral defects, frequently show these changes. Morgagni first described a number of these cases, and since his time many authors have confirmed the observation. In 1911, Elliott and Armour¹⁵ described an interesting case of a full term anencephalic fetus in which the suprarenals were about one-sixth normal size, and shaped exactly like those of a child 1 year old. Histologically the suprarenals showed complete development of the medulla, but absence of the cortex. Kern,¹⁶ Veit,¹²⁰ Meyer¹²¹ and Landau¹⁷ also reported that the hypoplasia of the suprarenals in these cases was limited to the cortex, which in most instances was not entirely absent. The medulla was either normal or hyperplastic when compared with control fetuses. Landau's explanation of the suprarenal cortical defect is that in anencephaly and hemicephal there is a hurrying forward, after the sixth month of fetal life, of the changes which ordinarily take place in the suprarenal cortex after birth (see involutionary changes described above). The result is a gland only slightly larger than that of the fifth month, the architecture of

119. Vincent, S.: Proc. Roy. Soc. London, series B, **82**:502, 1910.

120. Veit, B.: Deutsche med. Wehnschr. **38**:629, 1912.

121. Meyer, R.: Virchows Arch. f. path. Anat. **210**:158, 1912.

which is that of a normal gland one month or more postpartum, with a thin cortex and a fully developed medulla. In rare instances the cortex may be entirely missing. An enlarged thymus is sometimes found in these cases and may stand in regard to the suprarenals, in the relation of cause and effect, as suggested by the work of Marine¹²² in the rabbit and of Jaffe in the rat.¹²³ As yet the connection between the cerebral defect and the suprarenal changes is not explained.

Malposition of the Suprarenal.—Displacement of the suprarenal underneath the capsule of the kidney and liver is the most common malposition. In a strict sense the term should be applied only to structures containing both cortex and medulla, because aberrant cortical masses are usually accessories.

Appearance of the Suprarenals at Autopsy.—Changes in the suprarenals are found frequently post mortem. In milder forms, the cells in the innermost zone of the cortex separate and lose their staining power. In the severe forms, the medulla and the inner cortical layers may be converted into a hemorrhagic mass. Intermediate degrees of change occur between these two forms. The most advanced changes are found when death is due to acute infections.

General opinion is that these changes are essentially postmortem softenings, but the question of their origin is still far from settled. While Materna¹²⁴ does not deny the occurrence of some degrees of postmortem softening, he believes that most of these softenings have their origin ante mortem. He came to this conclusion because he frequently noted reparative changes in softened suprarenals, and he observed the most extensive postmortem lesions in those cases in which the suprarenal is known to undergo marked destructive changes during life (infections, intoxications and burns). According to this interpretation, the severe postmortem lesions are intensifications of antemortem or agonal changes. Some of the seemingly destructive changes may be artefact, because after death the suprarenal is easily injured by handling.

Changes in Suprarenals as a Result of Infections and Intoxications.—Edema is commonly found in the inner zone of the cortex, and may be so extensive as to change the size and weight of the gland. Congestion of the sinusoids, especially the zona fasciculata, also occurs frequently. Focal necrosis and hemorrhage of this zone are often associated with toxemias and intoxications, and in nonfatal cases regeneration occurs rapidly through active mitosis.¹²⁵ Concomitant with these pathologic changes is the more or less complete disappearance of cortical lipoids.

122. Marine (footnote 31, fourth reference).

123. Jaffe (footnote 31, second reference).

124. Materna, A.: Virchows Arch. f. path. Anat. **227**:235, 1920.

125. Mallory, F. B.: Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914.

Hemorrhage into the Suprarenals.—Massive hemorrhage involving both cortex and medulla has been noted frequently in the new-born, and may cause death in these cases. The hemorrhage may lead to complete destruction of the medulla, converting the glands into blood cysts. With extensive hemorrhage, the cortex sometimes ruptures, causing bleeding into the retroperitoneal tissues. In the new-born the pathogenesis of this condition is not understood, but in some cases it may result from an exaggeration of the physiologic degenerative changes which normally occur in the cortex soon after birth. In adults the lesion is frequently hemorrhagic infarction due to embolism.

Suprarenal Changes in Fatal Burns.—The significant pathologic change in these cases is a hemorrhagic destruction of the glands, and as the lesions are sometimes large, they may be the immediate cause of death. The pathogenesis is obscure. Kolisko¹²⁶ believed that the lesion was a hemorrhagic infarction, but Weiskotten¹²⁷ doubted this.

Amyloid Degeneration.—This is common in the suprarenal. The amyloid is deposited in the walls of the sinusoids; as it accumulates the parenchymal cells are gradually compressed. Most of the amyloid is deposited in the fasciculata.

Calcification of the Suprarenal.—A few cases have been reported in man. Newsam's¹²⁸ patient, a child, aged 2 years and 7 months, apparently healthy, suddenly became unconscious and died in fourteen hours. The autopsy showed calcification of the fascicular and reticular zones, with the formation of bone in the medulla. Victor¹²⁹ described a similar case, but without bone formation in the medulla. Kruse¹³⁰ reported an instance of bone formation in the suprarenal medulla of a monkey. Calcification is a common lesion in cats, Marine¹³¹ observing it in approximately 25 per cent of 257 animals. In a large series of rabbits, pigs, sheep and cattle he did not find the condition. He believes that in the cat it is associated with distemper.

Miscellaneous Pathologic Conditions.—Abscesses in pyemia have been described, but are uncommon. Tubercles are found constantly in miliary tuberculosis. In acquired syphilis gummas sometimes appear in the suprarenal, while in congenital syphilis the suprarenal shows focal necrosis, at times gummas and interstitial fibrosis, and fibrosis of the capsule. Spirochetes are demonstrated easily.

126. Kolisko, A.: *Viertljschr. f. gerichtl. Med.*, suppl. 1, **47**:217, 1914.

127. Weiskotten, H. G.: *Superficial Burns and Suprarenals*, J. A. M. A. **69**: 776 (Sept. 8) 1917.

128. Newsam, A. R.: *Rhode Island M. J.* **7**:35, 1924.

129. Victor, M.: *Ztschr. f. Kinderh.* **30**:44, 1921.

130. Kruse, H. D.: *Anat. Record* **28**:289, 1924.

131. Marine, D.: *J. Exper. Med.* **43**:495, 1926.

Changes in Suprarenal Constituents in Pathologic Conditions.—By histologic and chemical methods it has been determined that the cortical lipoids disappear rapidly in acute infections and intoxications, and more slowly in chronic infections. In anemias and as a result of severe hemorrhage they also disappear. On the other hand, in chronic cardio-renal disease, arteriosclerosis, cardio-valvular disease with decompensation, and conditions of inanition and wasting, the lipoids are increased. The disappearance of the lipoids does not take place evenly throughout the cortex. As exhaustion advances, focal areas still loaded with lipoids remain. Finally, only occasional cords of the fasciculata contain this substance which eventually disappears entirely. The mechanism by which the lipoids are removed from the cells is not known.

In diseased conditions the epinephrine store has not been found to be increased with certainty, not even in cases of hypertension with cortical tumors in which there is a suspicion that a relation between these conditions may exist. One of the difficulties in these estimations is that epinephrine tends to disappear rapidly after death. On the basis of the results of several investigators, there is no doubt that in the course of acute diseases the epinephrine store of the suprarenals diminishes, but its regeneration is rapid during convalescence.

ADDISON'S DISEASE

In spite of the great amount of data, the pathology of Addison's disease and the relative importance of the cortex and medulla in its production are not yet completely understood. Opinion on the latter question is divided. Karakascheff¹³² affirms that cortical destruction is the important lesion, while Wiesel¹³³ considers that the essential change is the degeneration or disappearance of chromaffin cells, either within or without the main glands. He believes that the cortical changes are of secondary importance. An understanding of the pathologic condition is further complicated by Lewin's¹³⁴ statement that in 12 per cent of a series of 561 cases compiled by him, the suprarenals apparently were normal. Perhaps Lewin overlooked such extrasuprarenal lesions as Wiesel described eleven years later, and, therefore, his statement is not as significant as it first appeared. On the other hand, destructive lesions of the suprarenals have been observed without the clinical syndrome of Addison's disease. These cases are usually explained on the basis that small amounts of cortical tissue have prevented the development of the symptom complex. In a small percentage of cases observers have described extensive alterations in the sympathetic system with and

132. Von Karakascheff, K. T.: Beitr. z. path. Anat. u. z. allg. Pathol. **36**: 401, 1914.

133. Wiesel, J.: Ztschr. f. Heilk. **24**:257, 1903.

134. Lewin, G.: Charité-Ann. **17**:536, 1892.

without lesions in the suprarenals. The changes were found chiefly in the semilunar ganglions and solar plexus which showed degeneration of the ganglion cells and nerve fibers, cellular infiltration, connective tissue proliferation and pigment deposition. Changes in the spinal cord in Addison's disease have been described, but are of doubtful significance. In spite of the foregoing observations, the important lesions in this disease must be sought in the suprarenals proper where the disturbance may be either a gross pathologic destruction of the gland or a physiologic insufficiency. Whether the cortex or medulla plays the important part cannot yet be settled. It has been shown in animals that the suppression of the epinephrine output from the main gland does not lead to any serious symptoms, and that the extramedullary epinephrine output is insignificant. Again chronic suprarenal insufficiency, due to cortical deficiency in animals, only vaguely resembles Addison's disease. The pigmentation has not been produced experimentally. Those who believe that cortical destruction is the important lesion must have seen cases at autopsy with completely destroyed medulla and with considerable areas of apparently uninvolved cortex. The cortex in these cases, though apparently normal histologically, may not be active functionally. Pathologists are hardly nearer a better understanding of the relation of the suprarenals to Addison's disease than they were in 1855. The entire subject should be reworked in the light of the present knowledge of experimental chronic suprarenal insufficiency, and of the vegetative nervous system.

From the etiologic point of view tuberculosis of the suprarenals has been found in at least 75 per cent of the cases. The lesion is usually bilateral, although occasionally only one gland is affected. Syphilis, hemorrhage, new growth and atrophy are among the other causes. Atrophy is considered by some the second most frequent lesion, and the condition is always bilateral.¹³⁵ Bittorf¹³⁶ compiled fifty such cases sonii: *Klinische und anatomische Untersuchungen*, Jena, Gustav-Fischer, 1908. which showed either small, normally shaped glands, or more atrophic glands consisting of fatty tissue which fused with the adjacent structures.

Elsässer¹³⁷ compiled 549 cases of Addison's disease, and in 17 per cent the lesion was isolated (primary) tuberculosis. This fact and the frequent observation of atrophy of the gland have led to the conception that disease attacks the suprarenal, causing the Addison syndrome, only when the suprarenals are subnormal from birth or congenitally. This view is further strengthened because it has been pointed out that status thymicolymphaticus is always present to some degree in patients with

135. Rolleston, H. D.: *Syst. Med.* (Allbutt), London 4:395, 1908.

136. Bittorf, A.: *Die Pathologie der Nebennieren und des Morbus Addi-*

137. Elsässer, P.: *Arb. a. d. Geb. d. path. Anat. Inst. zu Tübingen* 5:45, 1904.

Addison's disease. Star¹³⁸ first observed the presence of an enlarged thymus in a case of Addison's disease in 1895; following this Hedinger,¹³⁹ Wiesel,¹⁴⁰ Hart,¹⁴¹ and Pappenheimer¹⁴² confirmed the observation and reported many cases. The relation of status thymico-lymphaticus to small, deficient suprarenals has been well known for many years. If Addison's disease generally appears when the suprarenals are subnormal, the origin of the abnormality may be traceable to irregularities in the physiologic involution of the gland which takes place after the second week of postpartum life.

Attempts to explain further the individual symptoms of Addison's disease on insufficiency of either cortex or medulla are futile; the pigmentation of Addison's disease is an augmentation of the normal pigment deposit. As to the amount of epinephrine in the suprarenals in Addison's disease, Lucksch reports that it is less than in any other pathologic condition. This is only natural since the destruction of medullary tissue is great. Some observers have reported that with tuberculosis of one gland the amount of epinephrine in the intact gland may increase.

TUMORS OF THE SUPRARENAL CORTEX

Tumors arising from the cortex resemble more or less normal cortical tissue. There may be simple hyperplasia, adenoma formation, degenerated adenoma and true carcinoma (hypernephroma).

Hyperplasia.—This condition occurs frequently in the suprarenal cortex. The lesions pass through hypertrophy, hyperplasia which may be focal or diffuse, to adenoma formation. The condition arises as a result of an increased physiologic demand made on the gland, and is found with increasing frequency after early adult life. In focal or nodular hyperplasia the cells or cell groups are often larger than normal, and usually present the structure of zona glomerulosa.

Adenoma.—True suprarenal adenoma is not common. It is separated from nodular hyperplasia by its atypical structure and malignant tendencies. This tumor is either single, or multiple and bilateral, and forms yellowish or yellowish red masses, well circumscribed from the rest of the cortex. It often deforms or destroys the gland. True adenomas do not reach any great size, though some tumors weighing several ounces have been described, while retaining the adenomatous structure. Hemorrhage and central softening may occur. Rarely adenomas are surrounded by a definite capsule.

138. Star, P.: *Lancet* **1**:284, 1895.

139. Hedinger, E.: *Frankfurt. Ztschr. f. Path.* **1**:527, 1907.

140. Wiesel, J.: *Internat. Clin.*, series 15, **2**:288, 1905.

141. Hart, K.: *Wien. klin. Wchnschr.* **21**:1119, 1908.

142. Pappenheimer, A. M.: *J. M. Research* **22**:1, 1910.

Microscopically the cells may deviate considerably from the normal cortical cells. Irregular alveolar formations with lumina, some showing low papillary growths, have been described in benign adenoma.¹⁴³ These tumors do not produce metastases, but transformation into adenoma-malignum or into more atypical carcinoma is frequent.

Carcinoma or Hypernephroma.—These tumors make up by far the majority of the malignant tumors of the gland. They may be divided into adenocarcinoma (adenoma-malignum) and undifferentiated carcinoma. The adenoma-malignum resembles benign adenoma, but shows atypical areas of malignant character, and metastasizes. In the typical areas, alveoli are reproduced, and the cells are arranged orderly. Usually there is a considerable vascular stroma. Various secondary changes, due to hemorrhage and necrosis, result in the formation of giant cells, cysts and pigmentation.

The undifferentiated carcinomas or hypernephromas on histologic examination show large, granular, fatty cells in perivascular arrangement, or the cells may lose all resemblance to those of the suprarenal parenchyma, and appear as rounded or polyhedral cells, free from fat or glycogen. These cells grow diffusely or in small groups, rapidly penetrate the surrounding tissues, and produce bulky metastases.

That some of the so-called sarcomas of the suprarenal are highly atypical carcinomas appears possible.

The malignant tumors may be small or extremely large, occupying considerable areas of the abdominal cavity. Early and widespread extension and metastases are prominent characters, but bone metastases, which are seen frequently in kidney hypernephromas, are rare in suprarenal hypernephromas (carcinomas). Although variegated, the gross appearance of these tumors is, nevertheless, characteristic. The color of the tumor is yellow, but because of the tendency to edema and hemorrhage, it shows smooth whitish or translucent areas, and areas that are discolored brown or red. At all stages the tumor is soft, and central necrosis may yield cysts.

Extrasuprarenal Cortical Tumors.—A certain number of tumors of the kidney have the gross and microscopic appearance of the malignant cortical tumors of the suprarenal, and are supposed to arise from suprarenal rests included in the kidney, as first proposed by Grawitz.¹⁴⁴ There has been considerable controversy, since that time, as to whether these tumors really arise from suprarenal rests. In spite of all opposition to Grawitz's view, Ewing¹⁴⁵ believes that in their characteristic form,

143. Manasse, P.: *Virchows Arch. f. path. Anat.* **145**:113, 1896.

144. Grawitz, P.: *Arch. f. klin. Chir.* **30**:824, 1884.

145. Ewing, J.: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1919.

renal hypernephromas are distinct histologically from renal adenocarcinomas, and it seems that in a certain number of cases Grawitz's view is correct, particularly since extrarenal hypernephromas have also been described.

Tumors, the histologic structure of which suggests origin from suprarenal tissue, have been described as arising from the scattered accessory cortical rests, free or in organs. The tumors frequently resemble suprarenal adenomas so strongly, that in many cases there does not seem to be any doubt of their origin from cortical tissue, though in certain organs this is questionable. In general these tumors are well encapsulated, of large size, cystic or solid, light yellow and with a tendency toward necrosis and hemorrhage. They are very malignant.

TUMORS OF THE SUPRARENAL MEDULLA

Each of the three types of cells present in the suprarenal medulla may give rise to tumors.

Sympathoblastoma or Neuroblastoma.—These tumors arise from embryonic sympathetic cells (sympathogonia) of the medulla; they are malignant and apparently all are of congenital origin. They are rarely seen, except in infants and children. Histologically the cells are small and round with little cytoplasm. There is a tendency for them to arrange themselves in alveolar formations or in little rosette-like nests. These are embedded in fibrils, which with special stains prove to be neurofibrils. Other structures such as imperfect ganglion cells, axis-cylinder processes and glia fibrils can sometimes be discerned. In the metastases round cells are present with or without fibrillar material. The resemblance between these tumors and congenital sarcoma and lymphosarcoma has been pointed out by Kretz¹⁴⁶ and Wright.¹⁴⁷ Round cell sarcoma in the upper part of the abdomen or in the retroperitoneal region in infants, together with many embryonal and peculiar growths of the cervical, thoracic and peripheral nervous system, even without involvement of the suprarenal, are now classed with sympathoblastomas.¹⁴⁸

Ganglioneuroma.—These tumors are rare, and occur generally before the twentieth year. Nearly all are nonmalignant, although an occasional case showing local infiltrative growth has been reported. The origin is from mature sympathetic ganglion cells, and the tumor may attain a large size. Histologically the ganglion cells may be small and poorly

146. Kretz, R.: *Ergebn. d. allg. u. path. Anat.* **8**:532, 1902.

147. Wright, J. H.: *J. Exper. Med.* **12**:556, 1910.

148. Lambert, R. A.: *Proc. New York Path. Soc.* **17**:96, 1917. Wahl, H. R.: *J. M. Research* **30**:205, 1914. Dunn, J. S.: *J. Path. & Bact.* **19**:456, 1914. Ewing (footnote 145).

differentiated, or large and multipolar, with medullated and non-medullated nerve fibers. The tumors may also arise from the sympathetic ganglion cells outside the medulla. Herxheimer¹⁴⁹ compiled twenty-nine cases, only eleven of which originated in the suprarenal medulla.

Paraganglioma.—These tumors arise from cells which give the chromaffin reaction. Reports of them are rare. They occur usually in adult life, and because of their small size (from 1 to 2 cm. in diameter) and their benign character, they are probably overlooked frequently or else found accidentally. This tumor is sharply demarcated from the cortex. Its substance is soft, cellular and vascular, with areas of hemorrhage and necrosis. Histologically it is composed of small polyhedral cells with indistinct cell boundaries and large well stained nuclei. In some cases true tumor giant cells with one or more nuclei have been described. Often collections of small cells, evidently sympathogonia, are seen, while larger cells, approaching the type of sympathetic ganglion cells, have also been described. Pigment is irregularly distributed through the tumor, and the typical chrome reaction is obtained. These tumors occur not only in the medulla but also wherever chromaffin cells are found, and have been described in such widely separated places as the neck and the bifurcation of the aorta.¹⁵⁰

SUPRARENAL MELANOMAS

Lucksch¹⁵¹ described small, brownish benign tumors, occurring in adults, and composed of cortical cells, which contain pigment resembling melanin. He believed that these tumors were the source for malignant pigmented growths.

Primary malignant melanotic tumors of the suprarenal involving the whole gland were described by Davidsohn,¹⁵² Goldzieher,¹⁵³ Tuczek¹⁵⁴ and Maclachlan.¹⁵⁵ The tumors occurred in adults and were bilateral, and sometimes produced metastases. Tuczek derived the tumors from chromatophore cells of neuro-epithelial origin which are found on rare occasions in the medulla. In the metastases Davidsohn recognized the structure of the zona fasciculata and demonstrated epinephrine, deriving

149. Herxheimer, G.: Beitr. z. path. Anat. u. z. allg. Pathol. **57**:112, 1914.

150. Stangl: Verhandl. d. deutsch. path. Gesellsch., 1902. Monckberg, J. G.: Beitr. z. path. Anat. u. z. allg. Pathol. **38**:1, 1905. Kawashima, K.: Virchows Arch. f. path. Anat. **203**:66, 1911.

151. Lucksch, F.: Beitr. z. path. Anat. u. z. allg. Pathol. **53**:324, 1912.

152. Davidsohn: Centralbl. f. allg. Path. u. path. Anat. **20**:506, 1909.

153. Goldzieher, M.: Centralbl. f. allg. Path. u. path. Anat. **24**:397, 1913.

154. Tuczek, K.: Beitr. z. path. Anat. u. z. allg. Pathol. **58**:250, 1914.

155. Maclachlan, W. W. G.: J. M. Research **33**:93, 1915.

the tumor, as does Goldzieher, from the suprarenal cortical cells. Maclachlan believes that suprarenal melanomas arise from chromatophore cells of mesoblastic origin, which invade the suprarenal medulla. All agree that the pigment is melanin.

THE RELATIONSHIP OF SUPRARENAL TUMORS TO VARIOUS
PATHOLOGIC CONDITIONS

Sex Disturbances.—The occurrence of tumors of the suprarenal cortex in association with disturbances in development of the body and sex glands has been known for more than a hundred years. Glynn¹⁵⁶ collected most of the cases in the literature up to 1910, and added several of his own. His report shows that malignant epithelial tumors of the suprarenal cortex in children (hypernephroma, adenocarcinoma, adenoma-malignum and diffuse carcinoma) are most common in females, in whom they induce the development of male primary and secondary sexual characters at the expense of the female characters. In his compiled cases in young females, menstruation was usually absent, the clitoris was enlarged and some children had beards. In young males such tumors caused accentuation and precocious development of the primary and secondary male characters. In adult females the tumors led also to atrophy of the sex organs, with the development of the male secondary sex characteristics.

Benign cortical hyperplasia or adenoma formation of the main glands or of accessories is sometimes seen in pseudohermaphroditism. The hermaphrodites are usually of the female type, that is females with male external organs. Kidney and other extrasuprarenal hypernephromas do not disturb the sex characters.

Hypertension.—Neusser¹⁵⁷ first pointed out the association of suprarenal tumors with hypertension, in the absence of renal and vascular disease. Vaquez¹⁵⁸ formulated the theory that arterial hypertension was due to a hyperepinephrinemia. Oppenheimer and Fishberg¹⁵⁹ recently reviewed the cases in the literature and added one case of their own. Sixteen apparently authentic cases of chronic nonnephritic hypertension, with an anatomically demonstrated lesion of the suprarenal gland, exist in the literature. In eleven of the cases the tumor was in the cortex (multiple adenoma or hypernephroma). In five cases the tumor was in the medulla (paraganglioma). An increased epinephrine content of the blood has not been demonstrated in these

156. Glynn, E. E.: Quart. J. Med. 5:157, 1911.

157. Neusser, E.: Nothnagel's Spec. Path. u. Therap. 18:71, 1898.

158. Vaquez, H.: Tr. Cong. franc. de méd. 1904, p. 338.

159. Oppenheimer, B. S., and Fishberg, A. M.: Association of Hypertension with Suprarenal Tumors, Arch. Int. Med. 34:631 (Nov.) 1924.

cases. It would be unavailing, at this time, to speculate on the mechanism of the hypertension.

Von Recklinghausen's Disease.—Paraganglionic tumors and hyperplasia of the chromaffin cells of the medulla have been reported in several cases of cutaneous Von Recklinghausen's disease. The exact significance of these changes is unknown.¹⁶⁰

THE RELATION OF THE SUPRARENALS TO STATUS
THYMICOLYMPHATICUS

After Star pointed out that enlargement of the thymus and lymphoid tissues was a prominent feature of Addison's disease, there was abundant confirmation of his view. A relationship between the suprarenals and the lymphoid tissues is also established from experimental studies. Crowe and Wislocki¹⁶¹ observed thymic enlargement in four dogs of a series of twenty that survived subtotal suprarenal extirpation for from six weeks to two months in good condition. It has been shown further by Marine¹²² and by Jaffe¹⁶² that suprarenalectomy in rabbits and rats not only prevents thymic involution, but also causes active regeneration, even of the highly involuted organ. In young rats, before sexual maturity, suprarenalectomy is an added stimulus for the growth of the thymus.

It is now generally recognized that the suprarenal glands are hypoplastic in status thymicolymphaticus. The earlier writers, among them Paltauf,¹⁶³ Wiesel¹⁶⁴ and Hedinger,¹³⁹ believe that the lymphoid overgrowth in status thymicolymphaticus and Addison's disease is due to a primary injury of the chromaffin tissue, while Marine and Jaffe believe that both in chronic suprarenal insufficiency and in these diseases it is related to a cortical disturbance.

160. Stangl (footnote 149). Kawashima (footnote 150, third reference).

161. Crowe and Wislocki (footnote 31, fifth reference).

162. Jaffe (footnote 31, first, second and third references).

163. Paltauf, A.: Wien. klin. Wchnschr. **3**:172, 1890.

164. Wiesel, J.: Virchows Arch. f. path. Anat. **173**:103, 1904.

Notes and News

Death of Chester Elliott.—Chester Elliott, aged 38, assistant professor of pathology and acting head of the department, in the University of Colorado Medical School at Denver, died on Dec. 8, 1926, after a brief illness.

Death of Victor Babes, 1854-1926.—Babes was born in Vienna and became professor of pathologic anatomy and director of the pathologic and bacteriologic institute in the University of Bucharest. He made important contributions to pathogenic bacteriology, was one of the first to demonstrate the presence of the tubercle bacillus in the urine, and discovered the parasite of Texas fever, which in Roumania is known as epidemic hemoglobinuria. With Cornil he published one of the first complete textbooks on bacteriology, in 1886. He published an extensive monograph on leprosy, and described a small coccus in measles which may have been the same diplococcus as that recently cultivated by Tunncliffe from the blood and throat secretions of measles patients.

Richardson Pathological Laboratory of Queen's University, Kingston, Canada.—This building, which is connected closely with the teaching hospital of the university, contains an autopsy room accommodating fifty students, and is provided with an improved table, and air fans to remove odors. Refrigeration is secured by means of brine. The building also contains a large museum with preparation room, an amphitheater seating 100, a large class room and six laboratories and private rooms. The close connection between the laboratory and the hospital is found to be of great advantage.

The Hooper Foundation and the Research Laboratory of the National Canners' Association.—The Hooper Research Foundation of the University of California, under Dr. Karl F. Meyer, has been asked to take charge of the bacteriologic laboratory of a \$100,000 research plant, constructed by the American Can Company for the National Canners' Association. The new three story building in San Francisco will be devoted to the improvement of canning methods in the Pacific Coast states and Hawaii, and to the safeguarding of the public wherever products from the district are sold. The plant is completely equipped with chemical and bacteriologic apparatus. Among the more important items are a hydrogen-ion outfit, equipment for heat penetration studies, microscopes, electric ovens and furnaces, apparatus for thermal death rate determinations, a refractometer and an electric refrigerator. The university is particularly interested in studies of botulism. The State Fish Commission has arranged a \$15,000 fund, donated by fish canners, for special research in this branch of the industry.

Abstracts from Current Literature

Pathologic Physiology

BLOOD CLOTTING STUDIES IN HEMOPHILIA. C. A. MILLS, *Am. J. Physiol.* **76**:632, 1926.

In four hemophilic children there was a rich supply of thrombin in very fresh serum, but not any prothrombin which could be activated by cephalin. The thrombin disappeared rapidly, and was not replaced as in normal serum. The action of cephalin on recalcified citrate plasma, and its inability to act on hemophilic serum, led the writer to conclude that the peculiarity of hemophilic blood lay in the prothrombin factor. Increased antithrombin was not found in these bloods. Normally reacting prothrombin appeared after protein sensitization, as well as after peptone injection.

H. E. EGGERS.

THE INFLUENCE OF BURNS ON EPINEPHRINE SECRETION. F. A. HARTMAN, W. J. ROSE and E. P. SMITH, *Am. J. Physiol.* **78**:47, 1926.

Experimental burns in anesthetized cats caused an increase in epinephrine output, as evidenced by the dilatation of the completely denervated iris. This increase sometimes persisted for a few hours. In the suprarenals a depletion of epinephrine and of lipoids was found.

H. E. EGGERS.

THE RÔLE OF THE SUPRARENAL GLAND IN THE NATURAL RESISTANCE OF THE RAT TO DIPHTHERIA TOXIN. D. L. BELDING and L. C. WYMAN, *Am. J. Physiol.* **78**:50, 1926.

Between two and three weeks after the removal of the suprarenal glands rats become about two and one half times as susceptible to diphtheria toxin as normal animals. The deficiency apparently impairs the normal mechanism of the rat for the destruction or elimination of the toxin.

H. E. EGGERS.

EFFECT OF THE TOTAL REMOVAL OF THE LIVER ON DEAMINIZATION. J. L. BOLLMAN, F. C. MANN and T. B. MAGATH, *Am. J. Physiol.* **78**:258, 1926.

In dehepatized dogs demonstrable deamination absolutely was not found, as evidenced by the recovery of amino-acids in the blood, urine and tissues in amounts approximately equal to the anticipated urea output of normal animals, and by the recovery of injected amino-acids unchanged from the blood. Urea formation did not occur, and neither ammonia nor glucose was formed from the amino-acids.

H. E. EGGERS.

EFFECT OF LIGATION OF THE HEPATIC ARTERY ON CARBOHYDRATE METABOLISM. W. S. COLLENS, D. H. SHELLING and C. S. BYRON, *Am. J. Physiol.* **78**:349, 1926.

Ligation of the hepatic artery in dogs produced death by hypoglycemia in from fifteen to fifty hours, depending on the previously stored glycogen. In the tissues at death there was a total glycogen depletion. Administration of glucose prevented convulsions and prolonged life for several hours. The syndrome does not follow cutting the periarterial nerve plexus.

H. E. EGGERS.

INTERRUPTION OF PREGNANCY IN THE RAT BY THE INJECTION OF OVARIAN FOLLICULAR EXTRACT. MARGARET G. SMITH, Bull. Johns Hopkins Hosp. **39**:203, 1926.

It is possible to interrupt pregnancy in the rat by the injection of follicular extract during the first five days of pregnancy. As the pregnancy proceeds from the first to the fifth days, it becomes necessary to inject much larger amounts of the follicular extract in order to interfere with its course. From these observations it seems possible that there is at least a difference in function between the follicular secretion and that of the corpus luteum.

AUTHOR'S SUMMARY.

THE INTRACUTANEOUS SALT SOLUTION WHEEL TEST; ITS VALUE IN DISTURBANCES OF THE CIRCULATION IN THE EXTREMITIES. WALTER G. STERN and MILTON B. COHEN, J. A. M. A. **87**:1355, 1926.

In the absence of edema, the intracutaneous salt solution test is a simple, rapid and accurate method of determining circulatory deficiencies in the extremities. Sixty minutes or more is the normal disappearance time of the salt solution. In all instances in which clinical circulatory deficiency exists, the disappearance time is diminished; in the area just above the seat of a gangrene (existing or threatened), it is frequently as low as five minutes.

AUTHOR'S SUMMARY.

INTESTINAL CHEMISTRY: V. CARBOHYDRATES AND CALCIUM AND PHOSPHORUS ABSORPTION. O. BERGEIM, J. Biol. Chem. **70**:35, 1926. VII. THE ABSORPTION OF CALCIUM AND PHOSPHORUS IN THE SMALL AND LARGE INTESTINES. Ibid. **70**:51, 1926.

The addition of lactose to the diet, in concentration of 25 per cent, greatly increases the absorption of calcium and, to a less degree, of phosphorus. The effect is less pronounced with other sugars, and may be due to an increased lactic acid fermentation in the intestines with a resulting increased acidity of the intestinal contents.

There is a secretion of phosphates into the upper part of the gastro-intestinal tract of both normal and rachitic rats which may be a factor in promoting calcium absorption. In the rachitic animals, perhaps as a result of the low phosphate concentration in the blood, the calcium absorbed in the upper intestine is again excreted into the lower one.

ARTHUR LOCKE.

STUDIES IN CARBOHYDRATE METABOLISM: IX. CONTINUED INVESTIGATIONS INTO THE INFLUENCE OF INSULIN AND MUSCLE TISSUE ON GLUCOSE IN VITRO. C. LUNDGAARD and S. A. HOLBØLL, J. Biol. Chem. **70**:71, 1926. X. INVESTIGATIONS INTO THE OCCURRENCE OF INSULIN COMPLEMENT IN THE MUSCLES OF WARM BLOODED AND COLD BLOODED ANIMALS. C. LUNDGAARD, S. A. HOLBØLL, and A. GOTTSCHALK, *ibid.* **70**:79, 1926. XII. INVESTIGATIONS INTO THE PROPERTIES OF INSULIN COMPLEMENT, *ibid.* **70**:89, 1926. XI. INVESTIGATIONS INTO THE OCCURRENCE OF NEW-GLUCOSE IN THE COURSE OF THE FERMENTATION OF ALPHA-BETA GLUCOSE, *ibid.* **70**:83, 1926.

There may be an active principle in muscle tissue which, together with insulin, is capable of transforming alpha-beta glucose into "new-glucose." It is proposed to call this principle "insulin complement."

"Insulin complement" may be detected in the muscles of both warm and cold blooded animals.

It is not removed from muscle by extraction with water nor can it be detected in expressed muscle juice. It is destroyed by warming at 70 C. for two minutes.

"New-glucose" is not formed during the fermentation of glucose. The fermentation of glucose may be basically different, in its early stages, from the breaking down of glucose in the animal organism.

ARTHUR LOCKE.

NITROGEN METABOLISM IN THE CHICK EMBRYO. C. H. FISKE and E. A. BOYDEN, *J. Biol. Chem.* **70**:535, 1926.

Uric acid begins to accumulate in the allantoic cavity of the chick embryo shortly after the embryonic kidney establishes a connection with the allantois. The rate of uric acid excretion is somewhat proportional to the body rate although it may increase disproportionately on the eighth and eleventh days. The allantoic fluid contains, by the thirteenth day, an average of 15 mg. of uric acid, and by the nineteenth day, some 100 mg. of solid urate. The impermeability of the allantoic wall to urates and the low solubility of these salts make the latter ideally adapted to serve as the end-products of protein metabolism during the embryonic life of the chick. The nitrogenous excretions of the chick embryo are confined to the allantois.

ARTHUR LOCKE.

THE FATE OF SUGAR IN THE ANIMAL BODY: II. THE RELATION BETWEEN SUGAR OXIDATION AND GLYCOGEN FORMATION IN NORMAL AND INSULINIZED RATS DURING THE ABSORPTION OF GLUCOSE. C. F. CORI and G. T. CORI, *J. Biol. Chem.* **70**:557, 1926. III. THE RATE OF GLYCOGEN FORMATION IN THE LIVER OF NORMAL AND INSULINIZED RATS DURING THE ABSORPTION OF GLUCOSE, FRUCTOSE AND GALACTOSE. C. F. CORI, *ibid.* **70**:577.

Insulinized rats oxidize more of a given quantity of absorbed dextrose and deposit less glycogen than normal rats. Large doses of insulin tend to inhibit the deposition of glycogen in the liver, although an abundant glycogen deposition may occur in the muscles. The increased sugar oxidation in the insulinized animals occurs chiefly in the muscles.

ARTHUR LOCKE.

EXSANGUINATION-TRANSFUSION IN THE TREATMENT OF MERCURIC CHLORIDE POISONING. C. C. HASKELL, J. R. HAMILTON and W. C. HENDERSON, *J. Lab. & Clin. Med.* **11**:707, 1926.

The dose of 4 mg. of mercuric chloride intravenously is fatal to the great majority of dogs, unless there is reason to suspect faulty technic.

After the injection of 4 mg. of mercuric chloride per kilogram intravenously into dogs, the removal of large amounts of blood and the subsequent injection of a compatible blood from another animal or of a solution of bicarbonate of soda, 1 to 5 per cent, appears to have not the least value, as judged by the early death of the animals treated in this manner.

S. A. LEVINSON.

THE NUTRITIVE REQUIREMENTS OF GROWING CELLS. TREVOR B. HEATON, J. Path. & Bact. 29:293, 1926.

The author grew various tissues from the chick embryo in Drew's saline mixture instead of in clotted plasma. Pieces of skin, liver and intestine showed growth in the saline medium up to the eleventh day only. In addition to the thermolabile substance or trephone, considered necessary to the permanent life of tissues in vitro, the capacity of tissue cells for multiplication by cell division depends also on the presence of certain thermostable growth-promoting substances.

Heaton found that the requirements for growth of epithelium and fibroblasts are not the same. Two separate factors are necessary for the multiplication of fibroblasts and epithelial cells after the eleventh day of incubation. Both factors are present in embryonic tissue extract, in blood serum and in most, if not all, adult organs. The thermostable substance necessary for growth of epithelium is apparently identical with vitamin B, and the addition of yeast to the saline culture medium after the eleventh day caused abundant growth. The thermostable substance necessary for the multiplication of fibroblasts is soluble in water and alcohol, but less soluble and less stable than the vitamin B, and is absent from yeast. Growth of fibroblasts may occur in the absence of this substance when in the vicinity of growing epithelial cells. The growth of fibroblasts is not only not promoted by the presence of yeast in the medium but also is positively inhibited. This inhibiting substance is thermostable (up to 125 F.), and soluble in water and alcohol up to 75 per cent. This substance is not confined to yeast but is present in adult mammalian organs especially liver.

E. M. HALL.

THE INFLUENCE OF THE VASOMOTOR STATE OF THE PERIPHERAL BLOOD VESSELS ON THE LEUCOCYTIC CONTENT OF THE BLOOD. A. F. BERNARD SHAW, J. Path. & Bact. 29:389, 1926.

The distribution of leukocytes in peripheral blood is influenced by the vasomotor state of the peripheral veins (in rabbits) and "capillaries" (in man).

In vasoconstriction leukocytes accumulate in the vessels and are unequally distributed on opposite sides of the body. Vasodilation, mechanically produced, is accompanied by an immediate fall in the number of leukocytes which are equally distributed on both sides of the body.

Blood from a dilated vein has the same leukocytic value as arterial blood and therefore represents the condition existing in systemic blood.

The leukocytic content of peripheral arterial blood is not influenced by vasomotor changes.

The leukocytic alterations are not due to changes in blood concentration and are not accompanied by variations in the leukocytic formula.

In addition to the local accumulation of cells in the peripheral vessels under the influence of vasoconstriction there are "physiological oscillations" which are independent of vasomotor activity and are systemic in origin.

Under prescribed conditions the local application of cold (man) causes a rise in the leukocytes and heat locally applied (rabbit) a fall in the count.

AUTHOR'S SUMMARY.

CLINICAL AND EXPERIMENTAL STUDIES ON THE INSULIN-ADRENALIN ANTAGONISM.
BODEN, DETERMANN and WANKELL, Klin. Wchnschr. **5**:1761, 1926.

After injection of insulin there appears constantly a leukocytosis and a lymphopenia in the peripheral blood; after epinephrine hydrochloride, a lymphocytosis. In man insulin, like epinephrine hydrochloride, produces a rise in blood pressure in close conjunction with a rise of blood sugar. The blood pressure falls with epinephrine hydrochloride in hypoglycemia. Unlike epinephrine hydrochloride insulin by its action on the parasympathetic system causes a marked respiratory arrhythmia, detected by the electrocardiograph. The chronotropic, inotropic and dromotropic action of insulin on the frog's heart is negative; that of epinephrine hydrochloride positive. Insulin produces an increase of the tonus and the automatism of the isolated guinea-pig intestine; epinephrine hydrochloride, a decrease. The conclusion is that there are opposed hormonal actions of insulin and epinephrine hydrochloride which extend far beyond the carbohydrate metabolism to other organic functions.

J. D. WILLEMS.

BIOCHEMICAL INVESTIGATION OF THE URINE OF OLYMPIC ATHLETES. O. FLÖSSNER
and F. KUTSCHER, München. med. Wchnschr. **73**:1434, 1926.

The physical exertion of the athletes taking part in the Olympic Games caused in their urine the presence of large quantities of lactic acid, a decrease of hippuric acid and a marked increase of adenine, methylguanidine and phenylalanine.

J. D. WILLEMS.

THE EFFECT OF INSULIN UPON THE ACIDOSIS OF HYPEREMESIS. H. SEIDL, München.
med. Wchnschr. **73**:1471, 1926.

Ten hyperemesis gravidarum patients with ketonuria and acetonuria were given insulin injections. The acidosis immediately subsided. Later, hypoglycemia followed, with return of the appetite, gain in weight and complete recovery.

J. D. WILLEMS.

THE PATHOLOGY OF THE INHERITANCE OF ACNE. H. SIEMENS. München. med.
Wchnschr. **73**:1514, 1926.

In thirty-six pairs of single ovum twins, acne vulgaris was present in practically identical form, location and severity; in twelve pairs of two ovum twins there were marked differences. This was considered exact statistical proof of the inheritability of acne.

J. D. WILLEMS.

THE METABOLISM IN TYPHOID FEVER. E. LE BLANC and LUDWIG GMELIN, Ztschr.
f. d. ges. exper. Med. **52**:14, 1926.

Characteristic changes in the metabolism occur in typhoid fever: In the febrile period, marked increase, returning to normal or nearly normal with subsidence of the fever, followed by increase again in convalescence, with a gradual return to normal. This course takes place regularly but not in so marked a degree, even when a good state of nutrition without loss in weight is maintained, hence it may be looked on as produced by the infection itself. In relapses the same metabolic curve recurs as in the main attack. How the infection causes the change in the metabolism is a problem requiring investigation.

Pathologic Anatomy

XANTHOCHROMIA OF SPINAL FLUID IN NEW-BORN. C. O. KOHLBY, *Am. J. Dis. Child.* **32**:58, 1926.

The pale yellow to bright yellow of the arachnoid fluid in five new-born infants is ascribed to the admixture of blood with the fluid due to disturbances during labor.

JANICEPS ASYMMETROS, WITH THE REPORT OF A CASE. G. PACK and I. BERREY, *Am. J. Obst. & Gynec.* **11**:779, 1926.

Pack and Berrey describe a monstrosity born of normal parents after eight months' gestation. It consisted of a fusion of homologous twins, one being better developed than the other. The heads were almost completely united, one face with a single eye being in a rudimentary state. The chests were fused above the umbilicus. Malformations of the internal viscera were less frequent in the better developed fetus.

A. J. KOBAK.

SEBACEOUS GLANDS IN THE HUMAN NIPPLE. O. PERKINS and A. MILLER, *Am. J. Obst. & Gynec.* **11**:789, 1926.

Discrepant descriptions offered in several textbooks led Perkins and Miller to study the sebaceous glands in forty male and female breasts. The glands usually have from six to nine saccules, which enlarge several times during lactation. These are lined with polyhedral cells, the outermost layer being cuboidal. The saccules are surrounded by a vascular collagenous tissue, and the connective tissue surrounding the entire gland contains smooth muscle fibers. The glands are found only at the tip of the nipple and are not associated with hair follicles.

A. J. KOBAK.

A CONTRIBUTION TO THE BIOMECHANISM AND PATHOLOGY OF ECTOPIC PREGNANCY WITH A CONSIDERATION OF SOME OF ITS CLINICAL PHENOMENA. M. ROBINSON, *Am. J. Obst. & Gynec.* **12**:1 and 232, 1926.

Robinson's thesis is based on a study of 131 cases. Detailed descriptions of ten cases, each well illustrated and representing a different type of ectopic gestation, are given. From these he shows that ovular nidation in extra-uterine pregnancy simulates the normal uterine in the formation of a reflexa and in decidual reaction. Quantitative and qualitative insufficiency of the nidation bed is responsible for transforming it into a pathologic condition. Intracapsular hemorrhage due to opening of the blood vessels by the chorionic villi is the main cause for premature termination of the pregnancy. Robinson believes the lack of tubal peristalsis, due to conditions which interfere with peristalsis, to be the chief cause of ectopic pregnancy. Inflammatory and neoplastic conditions, frequently attributed as the chief cause, were absent in his series in many instances. The point of rupture in the majority of cases was usually opposite the site of placental implantation. The capsularis at this point is thinnest and very much stretched, whereas the greatest amount of hypertrophy and hyperplasia is to be seen at the placental area. Uterine bleeding is usually an indication of fetal death and of cessation of the inhibitory power of the corpus luteum over the endometrium.

A. J. KOBAK.

VASCULAR MECHANISM OF SPLEEN. W. L. ROBINSON, *Am. J. Path.* 2:341, 1926.

The fibromusculo-elastic capsule and trabeculae serve as a framework for the pulp which they divide into lobules. The pulp consists of a network of reticulo-endothelial cells whose protoplasmic processes unite with one another to form a labyrinthiform system of intercellular spaces through which the blood flows. The arterial circulation opens into the pulp by the flaring out and fusing of the end capillaries with pulp cells. The "ampullae of Thoma" are exaggerated pulp spaces. The ellipsoids have the same structure and cellular content as the pulp except that they are more compact. The intralobular veins have incomplete walls, having slitlike stomas which communicate freely with the pulp spaces. The ellipsoids and end capillaries are permeable to gelatin solutions. Gelatin solutions injected into the artery pass through the pulp before reaching the veins, except when a venous channel lies contiguous with the ellipsoid. In the latter case, the flow is through the interstices of the ellipsoid and stomas of the vein wall into the lumen of the vein. There are no direct communicating closed channels between arteries and veins other than the pulp spaces. The circulation of the spleen is open.

AUTHOR'S SUMMARY.

RENAL INFECTION IN PULMONARY TUBERCULOSIS. EDGAR M. MEDLAR, *Am. J. Path.* 2:401, 1926.

Renal tuberculosis is common in advanced pulmonary tuberculosis (twenty-two of thirty cases). In 75 per cent of the cases the lesions are cortical; they are hematogenous and bilateral, and may heal.

EXPERIMENTAL PRODUCTION OF GENERAL PERITONITIS. BERNHARD STERNBERG, *Am. J. Path.* 2:415, 1926.

Acute and healing peritonitis may be produced by the intraperitoneal injection of a twenty-four hour culture of colon bacillus with antiserum against the bacillus. Polymorphonuclear leukocytes predominate in the exudate and mononuclear leukocytes in the fixed tissues.

FORMATION OF GIANT CELLS IN TURTLE BLOOD CULTURES. MORTIMER COHEN, *Am. J. Path.* 2:431, 1926.

Only large mononuclear cells entered into the formation of giant cells by fusion in hanging drop cultures of turtle blood. The giant cells thus formed appear to behave like true cells.

HEMORRHAGIC PROCESSES IN THE THYROID GLAND. MAX BALLIN and P. F. MORSE, *Ann. Clin. Med.* 4:613, 1926.

Hemorrhages into the thyroid gland, resulting from direct trauma, rupture of thin walled cysts or hemorrhage around an infectious embolus, are frequently responsible for the sudden onset of toxic thyroid symptoms.

WALTER M. SIMPSON.

A NEW CASE OF FREE BALL THROMBUS ASSOCIATED WITH MITRAL STENOSIS. EUGENE B. POTTER, *Ann. Clin. Med.* 4:736, 1926.

Two cases in which a free ball thrombus was found in the heart were encountered as autopsy discoveries during a period of twenty months at the Pathologic Laboratory of the University of Michigan. Abramson reported the

first case (*Ann. Clin. Med.* 3:327, 1924). In Potter's case there was mitral stenosis, with auricular fibrillation, and embolism of the right popliteal artery, which made midhigh amputation necessary. At autopsy, the heart was found to be greatly enlarged. The left auricle was enormously distended, and contained two thrombi, one of which was entirely free. It measured 5.5 by 4.5 by 4 cm. The second thrombus was pedunculated and firmly fixed in the left auricular appendage. The mitral valve showed extreme stenosis, of the "button-hole" type. In the lower 4 cm. of the aorta was an obliterating embolus which extended into each common iliac artery.

WALTER M. SIMPSON.

GENERALIZED TELANGIECTASIA. S. WILLIAM BECKER, *Arch. Dermat. & Syph.* 14:387, 1926.

Seven cases are described. The literature is reviewed and the cases are tabulated. An outline for intensive study is presented in order to increase the interest in this condition.

THE BLOOD IN SMALLPOX DURING A RECENT EPIDEMIC. K. IKEDA, *Arch. Int. Med.* 37:660, 1926.

The blood of smallpox shows characteristic features which, if properly interpreted, are of definite diagnostic and prognostic value. The earlier the rise of the platelets, the sooner the approach of the desiccation period, prognosticating a shorter course of the disease. A definite leukopenia during the maculopapular stages indicates, as a rule, a mild discrete form. A progressive leukocytosis with an early high polynucleosis predicts a severe form. The higher the values, the more probable is the fatal outcome. The early appearance of normoblasts, basophilic stippling and polychromatophilia, without evident anemia, is an unfavorable sign. It invariably means the purpuric form of smallpox. Condensation and fragmentation of mature leukocytes are found only in the purpuric form of smallpox. They appear comparatively early in the primary type of purpuric smallpox and are usually accompanied by pathologic normoblasts, basophilic stippling and polychromatophilia, without visible anemia. A rapidly progressive, absolute lymphocytosis is a constant characteristic of purpuric smallpox. Scarlatinal and other exanthems, infectious purpura and toxic rash with petechiae, etc., can be definitely differentiated from the purpuric form of smallpox during its erythematous stage by the blood changes.

S. A. LEVINSON.

THE EFFECT OF LEAD AND RADIUM ON MATURE AND IMMATURE RED BLOOD CORPUSCLES. H. E. PEARSE, *Arch. Int. Med.* 37:715, 1926.

That erythrocytes, following chronic loss of blood, on standing several hours in Ringer's solution and after exposure to lead, have an increased resistance to laking by hypotonic salt solutions is confirmed. Lead produces a greater increased resistance of mature than of immature red blood corpuscles. Irradiation with radium emanation does not change the resistance of mature or immature erythrocytes. Lead and radium combined produce an effect midway between the effect of either alone. Radium hemolyzes more readily immature than mature red corpuscles while lead has just the opposite effect from radium. Irradiation of "leaded" erythrocytes greatly increases the amount of hemolysis over that by either agent alone. This effect is twice as great on "leaded" immature as on "leaded" mature red blood corpuscles.

S. A. LEVINSON.

CHRONIC ENDOCARDIDIS ASSOCIATED WITH DEFECTS OF THE SEPTUM VENTRICULORUM. C. ALEXANDER MCINTOSH, *Ann. Clin. Med.* 4:748, 1926.

McIntosh found at autopsy two defects in the pars membranacea of the septum ventriculorum just beneath the right anterior aortic cusp. The edges of the defects showed extensive sclerosis, while the mitral valve was the site of an old healed sclerosing mitral endocarditis, on which was superimposed an active recent vegetative process.

WALTER M. SIMPSON.

CEREBRAL BIRTH INJURIES AND THEIR RESULTS. F. R. FORD, *Medicine* 5:121, 1926.

It is not possible to reach any final conclusions about the exact limits of the group of cerebral birth palsies from the evidence available. The cases of spastic paralysis have been difficult to analyze, and in many cases a satisfactory neurologic examination has not been possible. Few patients with intracranial hemorrhage which survived could be found and these have not been followed long enough. The pathologic anatomy is meager and usually imperfectly described and studied. Before any clear understanding of the problems can be secured a large series of detailed pathologic anatomic studies must be made, with adequate clinical control. However, unsatisfactory as the material may be, certain impressions have been gained in the course of this investigation which it seems permissible to state in the form of tentative conclusions.

First, convincing evidence has been gathered that the congenital diplegias which constitute by far the largest group of infantile spastic palsies, as seen in the pediatrics department (235 of 280 in all), are not to be attributed to meningeal hemorrhage at birth, but are the result of various pathologic processes of intra-uterine origin. Four principal arguments support this conclusion. Meningeal hemorrhage is in at least half the cases unilateral, and when bilateral is almost always unequal on the two sides; cerebral diplegias are, with few exceptions, bilaterally symmetrical. The heads of patients with diplegia are usually either definitely microcephalic or slightly below normal size; meningeal hemorrhage causes rapid enlargement of the head. Children who are known to have intracranial bleeding at birth or in infancy do not develop cerebral diplegia. Lastly, the pathologic anatomy of true congenital diplegia is such that it cannot be reconciled with any theory of birth injury.

If the foregoing statements are correct it will be seen that cerebral birth injuries are rare rather than common, and the great mass of infantile palsies can no longer be lightly attributed to faulty obstetric procedures.

No final statement can be made about the relation of intracranial birth injury and chronic hydrocephalus. Undoubtedly, marked enlargement of the head may occur, with extensive bleeding into the ventricular system or subarachnoid spaces, but in most cases the blood is eventually absorbed without leaving any permanent obstruction to the circulation of cerebrospinal fluid. Possibly, in some cases, chronic hydrocephalus may result from plugging of the aqueduct by a blood clot or a bit of necrotic brain tissue, and organization of the obstruction. It seems likely that some children with large heads and congenital asymmetrical spastic paralyses owe their condition to birth injury. Intracranial hemorrhage is not uncommonly an accidental complication of congenital hydrocephalus because these infants have large heads and thin skulls.

The common diffuse meningeal hemorrhage which is not large enough to cause death apparently leaves no residuum in the overwhelming majority of cases. The real birth injuries to the brain are caused by the rarer intra-

cerebral hemorrhages and necroses, by depressed fractures with laceration of the brain and, undoubtedly, by some more or less encapsulated meningeal hematomas which compress and soften the cortex. All the evidence which the writer was able to collect seems to indicate that the true cerebral birth palsies are represented by the congenital hemiplegias, the monoplegias and the asymmetrical and unequal bilateral spastic paralyses. The children have large or normal heads, even in some cases asymmetrical heads. Their intelligence is on the average much greater than that of the diplegic patients. A history of birth injury may be obtained in a large percentage of such cases and the pathologic anatomy is quite consistent with the hypothesis of gross birth injury. Of course, it is realized that asymmetrical congenital defects of the brain are included with these cases and are probably clinically indistinguishable from them. Numerically this group, which the writer believes is principally composed of true cerebral birth palsies, is surprisingly small, just seventeen of 280 infantile cerebral palsies in the Harriet Lane Home records, if the five asymmetrical diplegias are excluded. These figures would mean that about 6 per cent of all infantile cerebral palsies are due to birth injury, but the group is not well enough defined to attempt any numerical estimation. Recurrent convulsions occur in about one-third or more of these cases, and it has already been concluded that about 2 or 3 per cent of all epilepsies seen in children are related to birth injury. Severe grades of mental defect are probably not related to birth injury with the exception of that type which develops in association with frequent convulsions. Tredgold estimates that not more than 1.5 per cent of patients suffering from dementia who die in asylums owe their condition to birth injury and it seems probable that these figures are approximately correct.

AUTHOR'S CONCLUSIONS.

TWO CASES OF STRICTURE OF THE BOWEL BY MISPLACED ENDOMETRIAL TISSUE.
T. MOUAT, Brit. J. Surg. 14:76, 1926.

In one case of stricture in the ileum 9 inches (23 cm.) from the ileocecal valve, there was an associated tubo-ovarian cyst on the right side. The other stricture was in the sigmoid and was associated with a ruptured tubo-ovarian cyst with many adhesions in the region of the left ovary. Microscopically, in both cases, endometrial tissue could be seen invading the muscular coats, but the lumen was not affected.

A. J. KOBAK.

CENTRAL PONTINE HEMORRHAGE. A. M. CRAWFORD, Lancet 2:173, 1926.

Sudden loss of consciousness, bilateral paralysis, contracted pupils and hyperpyrexia were present in both cases. In one case there was a large central hemorrhage into the pons, escaping on the right, inferior surface. In the other case the hemorrhage in the center of the pons had burst into the fourth ventricle, and blood was present in the whole ventricular system.

A CASE OF POLYARTERITIS ACUTA NODOSA. JOHN GRAY, J. Path. & Bact. 29:245, 1926.

A case of polyarteritis nodosa in a boy, aged 12, is reported. While sledging he had scratched his left knee and this was followed a week later by fever, swelling and pain. After an illness of nine weeks he died suddenly. Autopsy showed a large aneurysm (6 by $3\frac{1}{2}$ by $3\frac{3}{4}$ cm.) of the cystic artery, which had ruptured. There were many small aneurysmal dilatations of the smaller

arteries, especially of the intrahepatic branches of the hepatic artery and of the coronary arteries. The aorta, superior mesenteric and main part of hepatic arteries were normal.

Histologically all coats of the smaller arteries were affected. The vasa vasorum and the adventitia were prominent and dilated. There was a perivascular infiltration consisting of lymphocytes and polymorphonuclear leukocytes. The media showed patchy disappearance and thinning, with cellular infiltration and fatty degeneration. The elastic lamina was repeatedly interrupted. The intima showed mainly proliferative changes which often filled the lumen completely, at times forming small grayish nodules on the inner surface of the vessel.

Organisms were not found in the sections of the diseased vessels, but the onset and febrile course of the disease indicate an infectious process.

E. M. HALL.

FOREIGN PARTICLES, THE RETICULO-ENDOTHELIAL SYSTEM AND ANAEMIA. A. R. ELVIDGE, *J. Path. & Bact.* 29:325, 1926.

Intravenous injections of particles of quartz, of indian ink, of carmine or of trypan blue give rise to the appearance of nucleated red cells in the circulation of the rabbit ("blood crises"). The animals recover in the course of two weeks. The nucleated forms begin to appear about five hours after injection. Marked reduction in the red cell count does not occur during a "blood crisis."

Repeated injections cause a reduction in the red cell count. The degree of the anemia depends partly on the intervals between injections, partly on the doses given. A severe anemia may lead to the death of the animal.

Still more immature and embryonic types of nucleated red cells accompany the "blood crises" and anemia. They are most abundant at the peak of the "blood crisis."

Hemoglobin determinations show that the color index remains in the neighborhood of 1 during the course of the anemia.

Suspensions of silicic acid jet and ultramicroscopic particles of quartz were injected without result, which speaks against a silicon poisoning effect.

The phenomena in question are due rather to the physical than to the chemical nature of the substances injected. They exert their action on cells of the reticulo-endothelial system.

AUTHOR'S SUMMARY.

EXPERIMENTAL URIC ACID NEPHRITIS. J. SHAW DUNN and C. J. POLSON, *J. Path. & Bact.* 29:337, 1926.

The intravenous administration of from 0.5 to 1 Gm. of uric acid per kilogram in the form of lithium monourate to rabbits produces necrosis of distal segments of the renal tubules while the proximal segments are uninjured. The localization of the lesion appears to be determined by precipitation of the uric acid in the distal segments.

This renal lesion causes oliguria and diminution of the urea concentrating function, and there is marked retention of urea in the blood.

Continued administration of smaller doses (0.025 to 0.1 Gm. per kilogram) produces only minor results without cumulative effect.

AUTHORS' SUMMARY.

ATHEROMA OF THE AORTA. J. B. DUGUID, *J. Path. & Bact.* 29:371, 1926.

Atheroma is a condition brought about by impairment of the elasticity and pliability of the tissues of the intima.

The lesions, which are marked by the presence of fatty deposit, are the result of the diastolic recoil of the vessel causing the intima to form folds and the more prominent parts of these folds tend to separate themselves from the media.

Proliferation of fibrous connective tissue occurs in association with fatty deposits in the tissues of the intima and follows as a reinforcing or reparative measure where this separation takes place.

Hyperplasia of fibrous connective tissue tends to diminish the pliability of the intima, and if it is irregular in its distribution it is likely to lead to injury of the type described in 2, so that excessive proliferation may bring about further changes of the very nature it is designed to counteract.

Atheroma is the mark of a vicious and progressive cycle of such changes.

The variations in the appearances seen in atheroma are to be accounted for by the variation in the degrees of elasticity which may be present in different aortas, because the amount of damage depends largely on the extent of the excursion through which the tissues pass during the pulse wave.

Factors which tend to increase the range of pulse movement increase the liability to atheromatous changes, and of these factors the most important are hypertension with high blood pressure (hyperpiesis) and the strain caused by the focal fixation of the aorta by its branches.

The cicatricial fibrosis of syphilitic aortitis causes impairment of the intimal pliability, and this leads to atheromatous change at the affected parts.

Fibrosis cannot be demonstrated in the earliest stages of atheroma but edema and hyaline change can be seen which may cause considerable loss of pliability in the intima.

Early atheromatous changes and acute general toxemic conditions are united in their association with hyaline and edematous change of the aortic intima.

AUTHOR'S SUMMARY.

OBSTRUCTION OF ESOPHAGUS CAUSED BY PERSISTENT DUCTUS ARTERIOSUS. A. J. JEX-BLAKE, *Lancet* 2:542, 1926.

In a dog, gradual starvation and asphyxia resulted from the gradual contraction of the ductus arteriosus, because the esophagus and trachea passed between the ductus and the arch of the aorta.

HISTOLOGIC STUDY OF A CASE OF CUTIS VERTICIS GYRATA. F. CURTIS, *Ann. d'anat. path.* 3:529, 1926.

The lesion consists in an overgrowth of the scalp with the formation of large folds arranged in an orderly fashion, but not necessarily gyrate. Unna was first to designate it as "Cutis Verticis gyrata." Audry described it under the name of "pachydermie occipitale vorticellée." The outstanding feature is an enormous thickening of the scalp, with fibrous hyperplasia. The tissue is hard, and scantily covered with hair. The disease is congenital, and progresses slowly. In one instance the patient was 67 years old. The lesion has been regarded as a giant nevus. Curtis produces evidences to show that it concerns not only a nevus but also a real tumor developed on top of a nevus, which he regards as a nevofibroma.

B. M. FRIED.

RÔLE OF EXCRETORY SYSTEM OF PANCREAS IN THE GENESIS OF LANGERHANS ISLANDS. PAUL HICKEL and JEAN NORDMANN, *Ann. d'anat. path.* **3**:587, 1926.

Numerous investigators have attributed a predominant rôle in evolutive processes of the pancreas to the secretory system. Thus, in the genesis of the islands of Langerhans the acini have been considered as the point of origin for these structures. Hickel and Nordmann produce evidence that the excretory system plays the dominating rôle in the repair of the glandular system. They affirm that in man the islands of Langerhans during life are formed at the expense of the excretory system, the centro-acinar cell proliferating to form islands. This transformation is not direct. It occurs only after the acinar cell has undergone profound changes provoked for instance by a sclerotic process. On the other hand, the possibility of a transformation of an island of Langerhans into an acinus is denied.

FIBRO-GÉODIC-OSTEITIS. RECKLINGHAUSEN'S DISEASE OF THE BONE. CHAUVEAU, *J. ann. d'anat. pathol. et d'anat. méd.-chir.* **3**:243 (March) 1926.

By "Maladie osseuse de Recklinghausen's" Chauveau means a disease characterized by a fibrous transformation of the bone marrow. This process leads finally to a dissolution of the medullary tissue with the formation of cavities (géodes). The bone itself undergoes rarefaction. Spontaneous fractures in these cases, followed by deformities and functional disturbances, are frequent. The article is a comprehensive review with a rich bibliography on the subject.

B. M. FRIED.

FORMATION OF XANTHOMA CELLS IN THE BREAST. E. LOBECK, *Arch. f. klin. Chir.* **141**:540, 1926.

Lobeck found xanthoma cells in nineteen (16 per cent) of 117 breasts which had been amputated for mastitis, carcinoma or benign tumor. The blood cholesterol was increased. Xanthomatous connective tissue cells were found only when the cholesterol content was more than 200 mg., with 180 mg. as a normal value. The xanthomatous connective tissue cells were true xanthoma cells; the xanthomatous epithelial cells, pseudoxanthoma cells.

PATHOLOGIC-ANATOMIC CHANGES IN BILHARZIASIS MANSONI. P. H. J. LAMPE, *Arch. f. Schiffs-uno Tropa-Hyg.* **30**:484, 1926.

Changes due to bilharziasis or schistosomiasis are frequently found at post-mortem examinations. In severe cases the changes are common in the lungs, and here, as well as in the liver, peritoneum, intestine, heart and lymph nodes, the changes are difficult to distinguish from those of miliary tuberculosis or carcinomatosis. In the centers of the nodular formations, the characteristic eggs may be seen. By special search, the fluke may be found in the blood of the portal vein and liver, and also of the lungs. In connection with this report by Lampe, see Plaut, Alfred: *Bilharzia Infection in Apparently Normal Appendix* (*ARCH. PATH.* **1**:711 [May] 1926).

A CONTRIBUTION TO THE HISTOGENESIS OF HEPATIC TUBERCLES. H. SCHLEUSSING, *Beitr. z. Klin. d. Tuberk.* **63**:317, 1926.

The first manifestation of tubercles in the liver is the appearance of small pale patches, which show microscopically different stages of degenerative changes in the specific liver cells, while the general structure of the tissue

is still recognizable; the nuclei and protoplasm stain poorly. The capillaries are normal at this stage. Karyolysis in the center of the focus follows and sometimes hyperchromatosis of the nuclear membrane. Alterations of the protoplasm, such as fat and lipoid infiltration and finally necrosis, appear later. Connective tissue cells and the vascular system are affected later in a similar way, particularly the endothelial cells. The author believes that in the histogenesis of tubercles the first stage is always an alteration of the tissue cells before reactive processes such as exudation and proliferation occur.

MAX PINNER.

FAT GIANT CELLS IN ERYTHROBLASTOSIS. ALFRED PLAUT, *Centralbl. f. allg. Pathol. u. path. Anat.* **38**:259, 1926.

In the axillary fat of the new-born with fetal hydrops and erythroblastosis reported by Bullard and Plaut (*Arch. Pediat.* **43**:292, 1926), were found large fat giant cells. The average diameter was from 40 to 80 microns, the largest being 170 microns, and the average number of nuclei was twelve. The protoplasm stained intensely with eosin. As paraffin embedding has been used, reactions for fat could not be carried out. In fourteen other new-born infants no giant cells were found. Were the giant cells in the case studied connected with the erythroblastosis? The changes in the body—general edema, erythroblastic and myeloblastic tissue in many organs, anemia, hypertrophy of the heart—corresponded to those of the type of fetal hydrops described by Schridde in 1910 and of which some twenty-five instances are recorded. As observed by Virchow and by Schridde in such cases, the thymus was small.

AUTHOR'S SUMMARY.

PSEUDOLEUKEMIA, LYMPHOSARCOMA, LYMPHOGANULOMA AND THEIR VARIOUS RELATIONSHIPS. S. M. ZYPKIN, *Folia haemat.* **32**:33, 1925.

The majority of diseases of the bloodforming organs are related. These organs react to irritants in three stages, if the irritation is continued and sufficiently severe. These three stages are called the pathologic triad. In the order of their occurrence they are: (1) degeneration of parenchyma, (2) proliferation of parenchyma, (3) proliferation of stroma. Thus, the aleukemias (pseudoleukemias) with their leukemic phases, together with the lymphosarcomas, represent the second stage. Hodgkin's disease, in its early or cellular form, also belongs in this stage, while its late or scirrhus form typifies the third stage. Embryonalization of both stroma and parenchyma in these conditions is considered one of the chief reasons for their relative malignancy. In the more benign diseases of the lymphatic and hematopoietic organs the irritation is more transitory, and this embryonalization does not occur. The term "pseudoleukemia" should apply only to the aleukemias. Banti's disease is a pseudoleukemia in which the splenic fibrosis represents a third stage, while the cellular marrow belongs to the second stage. Previously reported and new cases, many of which are transitional in type, are cited in support of these interpretations.

C. J. WATSON.

CONCERNING THE MORPHOLOGIC FEATURES IN INFLAMMATION IN LEUKEMIA. KURT BICKHARDT, *Folia haemat.* **32**:83, 1925.

The author discusses four cases of leukemia, complicated by acute inflammatory processes. Two of these were chronic lymphatic, one chronic myelo-

genous and one a stem cell, or myeloblastic, leukemia. In the lymphatic leukemia the inflammatory exudates consisted chiefly of lymphocytes (from 90 to 95 per cent). In the case of chronic myeloid leukemia the inflammatory exudate was composed chiefly of young cells of the myeloid series, while in the myeloblastic leukemia, complicated by pneumonia, the alveolar exudate was composed chiefly of myeloblasts. It is concluded that in exudates in leukemia the leukocytes peculiar to that disease take the place of the normal leukocytes.

C. J. WATSON.

HISTOLOGIC STUDY OF A CASE OF MYELOID LEUKEMIA, WITH MEASUREMENT OF THE MITOTIC ANGLES. SVEND PETRI, *Folia haemat.* **32**:103, 1926.

After death from chronic myelogenous leukemia in a man, aged 61, the bone marrow was similar to that of pernicious anemia, in that there was marked erythropoietic activity. With Ellermann's technic it was possible to recognize early, nongranular forms, some of which were pregranulocytic myeloblasts, while others are spoken of as erythrogonia.

Measurement of the angles of mitotic figures in fifty neutrophile myelocytes and forty myeloblasts gave an average reading of 68 degrees, while with the so-called erythrogonia (forty measurements) the average reading was only 22 degrees. A special instrument called the goniometerocular was used in measuring these angles.

C. J. WATSON.

A CASE OF ALEUKEMIC MYELOSIS WITH THE CLINICAL PICTURE OF MIKULICZ' DISEASE. L. S. HANNEMA, *Folia haemat.* **32**:116, 1926.

In a boy, aged 7 years, the parents first noticed an increasing swelling of the left eyelid. Later the right eyelid became swollen in a similar manner. The salivary glands and several cervical nodes were enlarged. The spleen and liver in time became definitely palpable. The leukocytes ranged from 3,200 to 7,000, while hemoglobin and red blood cells decreased progressively, and just before death the hemoglobin was 20 per cent. The first examination of the smears showed 60 per cent polymorphonuclear neutrophils, 2 per cent myeloblasts, 1 per cent myelocytes, 2 per cent undefined and 35 per cent of lymphocyte-like forms which were classified as micromyeloblasts of the type first described by Naegeli. During successive examinations this type of cell increased markedly in number. The last smears showed 75 per cent of these forms with only 12 per cent neutrophils. A minority of the micromyeloblasts gave a positive oxydase reaction.

At necropsy, there was enlargement of the lymph nodes, spleen and liver. Several leukemic foci were noted in the kidneys. The lachrymal and salivary glands were diffusely and markedly enlarged, and the normal structure of these organs was completely obliterated by round cells slightly larger than lymphocytes, some of which gave a positive oxydase reaction. These were all thought to be myeloid in origin. The author points out that the case reported by Mikulicz presented simply enlarged lachrymal glands, and he also mentions that this and subsequent cases of the same type were all lymphatic aleukemias, while the present instance seems to have been definitely myelogenous. With these differences, he asks whether the case now reported is to be included as an instance of so-called Mikulicz disease.

C. J. WATSON.

BLOOD CHANGES IN PNEUMONIA. ANTON HITTMAYER, *Folia haemat.* **32**:129, 1926.

In a man, aged 28, ordinarily strong and healthy, with croupous pneumonia of moderate severity and recovery by crisis, the blood changes were marked, many young lymphoid and myeloid forms appearing during the febrile stage; endothelial cells, young monocytes, histiocytes and irritation forms were noted, and giant neutrophils were seen frequently.

Arneth has reported giant neutrophils in five cases of pneumonia; nearly all these neutrophils showed marked toxic granulation.

A frail girl, aged 19, developed a rapidly fatal postinfluenzal pneumonia. Blood changes were scanty, consisting of toxic granulation of neutrophils, increased lobulation of monocyte nuclei and a slight increase in eosinophils.

From these two cases, the author concludes that the severity of the infection and the blood picture do not run parallel.

C. J. WATSON.

THE DETERMINATION OF INTRACRANIAL HEMORRHAGE IN THE STILLBORN WITHOUT AUTOPSY. L. SCHOENHOLZ, *Klin. Wchnschr.* **5**:1775, 1926.

By jugular injection with minium (red lead) at body temperature all the blood vessels of the brain and cranium are easily filled, so that by stereoscopic roentgenograms minute extravasations can be definitely localized.

J. D. WILLEMS.

HEMORRHAGE INTO THE PARATHYROIDS IN INFANTS AND SMALL CHILDREN AND THE SPASMOPHILIA PROBLEM. FELIX DANISCH, Frankfurt. *Ztschr. f. Path.* **33**:380, 1926.

Erdheim, 1906, described three cases of infantile tetany in which there was old hemorrhage in the parathyroids. He considered this the etiologic factor in the convulsions of the tetany. Other investigators since that time have obtained contradictory results. Grosser and Betke, in sixteen cases of tetany, found hemorrhage in four cases in one or more of the parathyroids. Hartwich, in twelve cases, found hemorrhage in only one.

Danisch found fresh or old hemorrhage, blood cysts and blood pigment in the parathyroids of infants and children up to 2½ years of age remarkably often, in over 30 per cent of cases. There is a tendency for the blood to become encapsulated because of a peculiar palisade arrangement of the parenchyma cells. The microscopic picture indicates that the bleeding is by diapedesis. There is no evidence that the bleeding is the result of an inflammatory process. The hemorrhage may be traced often to birth trauma or intrapartum asphyxia, but almost an equal number of cases occur later. Fourteen cases of bleeding into the parathyroids are cited. It does not appear that any active organ destruction goes on in the greater number of cases of hemorrhage. In ten cases of rachitis, eight did not show parathyroid hemorrhage. In two cases, Danisch found unmistakable bleeding in single organs which could not in any way be connected with the pathogenesis of spasmophilia. In only one case was there marked destruction of the parenchyma due to hemorrhage. Manifest tetany was present in this case. This was really a hypoparathyroid tetany similar to the postoperative tetany.

E. M. HALL.

THE IMPORTANCE OF STRUCTURE AND FUNCTION OF THE GASTRIC MUCOUS MEMBRANE FOR THE DEVELOPMENT OF CHRONIC GASTRIC ULCERS. F. BUCHNER and C. RUF, Frankfurt. *Ztschr. f. Path.* **33**:406, 1926.

The importance of the mucous membrane with its peculiar glands and their distribution in the development of gastric ulcers is emphasized. The authors believe that the secretory function plays a rôle as important perhaps as the anatomic function of the gastric musculature as held by Aschoff. Gastric ulcers develop commonly (25 per cent) in the beginning part of the pylorus. Buchner and Ruf explain that the active gastric juice coursing over a sensitive mucous membrane may be responsible. The distribution of chronic ulcers in the radiations of the gastric pathway also corresponds with the distribution of the pyloric mucosa. The ulcers of the duodenum develop in the majority of cases in the thick under half of the pylorus where the concentration of the active digestive juice is strongest for the duodenum. The postoperative ulcer of the jejunum develops with predilection, in those cases in which there was a preoperative duodenal ulcer. The sensitiveness of the intestinal mucosa which favored the duodenal ulcer favors also the jejunal ulcer.

E. M. HALL.

TRAUMATIC PERFORATION OF HEALTHY APPENDIX. R. GUTZEIT, *Zentralbl. f. Chir.* **53**:1943, 1926.

A kick by a horse caused rupture of the appendix in a boy, aged 14 years. This was the only intestinal injury. Symptoms of peritonitis set in immediately. An operation was performed and recovery ensued.

Pathologic Chemistry

HEMATOPORPHYRINURIA. PHILLIP E. ROTHMAN, *Am. J. Dis. Child.* **32**:219, 1926.

Two cases with classic complications are reported. Spectroscopically, both urines showed hematoporphyrin. A boy with polyneuritis died suddenly in the second attack. Autopsy revealed a generalized hyperplasia of the lymphatic structures with a persistent thymus. The spleen was enlarged but did not show any iron or evidence of phagocytosis. Hydroa-estivale was associated with the second case until the child was 8 years old. Pigmentation of the skin and discoloration of the urine persisted.

W. P. BLOUNT.

DIRECT AND INDIRECT DETERMINATIONS OF URIC ACID IN HUMAN BLOOD. G. W. CLARK and A. A. DE LORIMER, *Am. J. Physiol.* **78**:368, 1926.

Using Benedicts' method for direct, and that of Bullmer, Eagles and Hunter for indirect, determination of blood uric acid, it was found that incubation, inactivation with subsequent incubation or standing at room temperature for several days resulted in a definite but limited loss of the color producing substances determined as uric acid by the direct method. Refrigeration was effective in preventing this loss. The indirect method, on the other hand, yielded constant results in these conditions. From the fact that separation and separate determination of the color producing substances into two groups failed in many cases to give a combined value equal to that obtained by the direct method, it is concluded that at least three groups of color-producing substances are concerned here.

H. E. EGGERS.

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THE EFFECT OF CARBON TETRACHLORIDE AND ALCOHOL ON THE ACID-BASE BALANCE OF THE BLOOD. P. D. LAMSON and R. WING, *J. Biol. Chem.* **69**:349, 1926.

The inhalation of carbon tetrachloride in concentrations sufficient to produce deep anesthesia produces only a slight fall in the p_H and carbon dioxide content of the plasma, which is in no way comparable to that occurring after ether or chloroform. The oral administration of carbon tetrachloride and alcohol (together) produces no change in the acid base balance except in the terminal stage of intoxication and considerably after the appearance of symptoms. Alcohol alone may produce a slight alkalosis.

ARTHUR LOCKE.

AN IMPROVED PROCEDURE FOR THE EXTRACTION OF THE OVARIAN HORMONE AND SOME CHEMICAL PROPERTIES OF THE PRODUCT. J. O. RALLS, C. N. JORDAN and E. A. DOISY, *J. Biol. Chem.* **69**:357, 1926.

An improved method is described for the preparation of ovarian hormone from hog liquor folliculi. The product is virtually free from cholesterol and has a potency of from 25 to 40 rat units per milligram of solids.

ARTHUR LOCKE.

THE DETERMINATION OF PROTEIN IN SPINAL FLUID, WITH A NOTE ON THE INCREASE IN PROTEIN IN THE SPINAL FLUID IN TYPHUS FEVER. S. M. LING, *J. Biol. Chem.* **69**:397, 1926.

The protein content of the spinal fluid, as estimated by the color reaction with Wu's phenol reagent, may become definitely increased in typhus fever.

ARTHUR LOCKE.

THE ISOLATION AND CRYSTALLIZATION OF THE ENZYME UREASE. J. B. SUMNER, *J. Biol. Chem.* **69**:435, 1926.

Octahedral crystals of globulin having a urease activity of 100,000 units per gram separate from dilute alcohol and acetone extracts of fat-free jack-bean meal on standing at from 2 to 2.5 C. The preparations are sensitive to denaturation.

ARTHUR LOCKE.

THE TRANSPORT OF OXYGEN AND CARBON DIOXIDE BY SOME BLOODS CONTAINING HEMOCYANIN. A. C. REDFIELD, T. COLLIDGE and A. L. HURD, *J. Biol. Chem.* **69**:475, 1926.

Hemocyanin functions in the transport of the respiratory gases in the blood of certain invertebrates in accordance with the same physicochemical principles as hold for hemoglobin. It binds oxygen at such low tensions as to enable the blood to act as a sort of vacuum system, capable of utilizing the minute amounts of oxygen available in deep sea water.

ARTHUR LOCKE.

THE LACTIC ACID CONTENT OF CEREBROSPINAL FLUID. J. GLASER, *J. Biol. Chem.* **69**:539, 1926.

The lactic acid content of normal cerebrospinal fluid varies from 11 to 27 mg. per hundred cubic centimeters, a range which is from 4 to 40 per

cent less than that of the blood. The values may be normal or lower in cerebrospinal syphilis, and increased in nonsyphilitic meningitis, brain abscess, brain tumor and xanthochromia.

ARTHUR LOCKE.

THE SO-CALLED OXYGEN CONTENT OF METHEMOGLOBIN. J. B. CONANT and N. D. SCOTT, *J. Biol. Chem.* **69**:575, 1926.

Methemoglobin does not contain available oxygen. It is a ferric iron compound, while hemoglobin, oxyhemoglobin and carboxyhemoglobin are compounds of ferrous iron.

ARTHUR LOCKE.

THE RECRYSTALLIZATION OF UREASE. J. B. SUMNER, *J. Biol. Chem.* **70**:97, 1926.

The crystalline preparation of urease previously reported may be recrystallized from a chilled acetone buffer mixture without variation in purity.

ARTHUR LOCKE.

THE LIBERATION OF ADSORBED SUBSTANCES FROM THE PROTEINS: II. THE EFFECT OF ADDITION OF SODIUM OLEATE TO WHOLE BLOOD FILTRATES. S. M. ROSENTHAL, *J. Biol. Chem.* **70**:129, 1926.

By the addition of 25 mg. of sodium oleate per cubic centimeter of whole blood, it is possible to increase the nonprotein nitrogen in the blood filtrates from 20 to 55 per cent. This increase is due to the liberation of nonprotein nitrogenous substances which ordinarily remain attached to the proteins and do not appear in the filtrates.

AUTHOR'S SUMMARY.

THE COLORIMETRIC ESTIMATION OF CHOLESTEROL AND LECITHIN IN BLOOD IN CONNECTION WITH FOLIN AND WU'S SYSTEM OF BLOOD ANALYSIS. G. M. DE TONI, *J. Biol. Chem.* **70**:207, 1926.

The protein precipitate obtained in the Folin-Wu system of blood analysis is washed, dried and extracted with hot chloroform, and the cholesterol and lecithin content of the extract estimated by the Liebermann and Whitehorn methods. The procedures are given in detail.

ARTHUR LOCKE.

AMMONIA AND BLOOD SUGAR. A. A. HORVATH, *J. Biol. Chem.* **70**:289, 1926.

An increase in blood sugar concentration may offset an increase in ammonia concentration by favoring the formation of urea. This conclusion is based on the observation that dextrose addition to blood filtrates may result in increased urea nitrogen values and that the subcutaneous injection of ammonium carbonate or urease may produce hyperglycemia.

ARTHUR LOCKE.

THE LABILITY OF THE SULPHUR IN CYSTINE DERIVATIVES AND ITS POSSIBLE BEARING ON THE CONSTITUTION OF INSULIN. E. BRAND and M. SANDBERG, *J. Biol. Chem.* **70**:381, 1926.

It is not established that the labile sulphur group is responsible for the physiologic activity of insulin. The loss of this activity following treatment with alkalis may involve other factors than the removal of sulphur. The intravenous injection of cystine derivatives, in which the sulphur group is even more labile than in insulin, is without effect on the blood sugar level of fasted rabbits.

ARTHUR LOCKE.

THE SUGAR IN URINE AND IN BLOOD. O. FOLIN and A. SVEDBERG, *J. Biol. Chem.* **70**:405 1926.

The blood contains a variable quantity of an unknown, fermentable sugar which is not glucose nor a disaccharide. The new sugar reaches its highest concentration in the blood of diabetic patients with high total sugar values. The concentration is decreased following the injection of insulin, either decreased or increased after injection of epinephrine hydrochloride and unaffected by oral administration of dextrose. The usefulness of the Folin copper method for the determination of sugar in blood and in normal urine is discussed, and revisions are proposed.

ARTHUR LOCKE.

BLOOD CHANGES IN ACUTE MERCURIC CHLORIDE POISONING. J. M. LOONEY, *J. Biol. Chem.* **70**:513, 1926.

The marked increase in blood nonprotein nitrogen observed during the coma of acute mercurial poisoning is not paralleled proportionately by increase in amino acid, nitrogen and uric acid.

ARTHUR LOCKE.

CHRONIC ALCOHOL POISONING. E. and I. KEESER, *Arch. f. exper. Path. u. Pharmacol.* **113**:188, 1926.

The blood chemistry of fifty-one persons with chronic alcohol poisoning or delirium tremens was studied. The results showed changes in fat metabolism with loss in phosphates, soaps and cholesterol; an increase in indican showing destruction of tryptophan, and an increase in acetone bodies. These changes may influence ferment activity and thus disturb protein metabolism.

ALKAPTONURIA. G. KATSCH and G. STERN, *Deutsches Arch. f. klin. Med.* **151**:329, 1926.

The serum of alkaptonuric patients contains a nonprotein substance that inhibits the oxidation by the serum of homogentisic acid. In acetonemia, alkaptonuria disappears, perhaps because of the binding of this substance or of a different way of cleavage of amino acids.

THE PRESENCE OF HEMATIN IN THE BLOOD OF MAN AND ANIMALS. K. BINGOLD, *Klin. Wchnschr.* **5**:1550, 1926.

Hematin appears in normal blood kept in sterile containers at body temperature for from twenty-four to forty-eight hours, but never normally intravascularly. Hematinemia occurs in pernicious anemia, acute yellow atrophy of the liver, malaria, gas bacillus sepsis and poisoning due to phenylhydrazine, toluylene diamine, dinitrobenzol, acetic acid and a few other substances. The presence or absence of hematin in the blood stream is thus of differential diagnostic value, and the determination can be made quantitatively by the spectroscope. A relationship between pernicious anemia and toxic processes is also suggested. Twelve years of clinical observation were followed by animal experiments. No hematin was found in the blood stream of dogs, rats, guinea-pigs and rabbits, following the production of a secondary anemia by bleeding. Phenylhydrazine, toluylene and gas bacillus emulsions produced not only hematinemia but also blood changes somewhat like those of pernicious anemia. The pigment disappeared when the poison was stopped, except in

splenectomized animals. A methemoglobinemia and sulphohemoglobinemia appeared only occasionally. The conclusion is that the appearance of blood pigments, especially of hematin, is associated with endogenous or exogenous toxins in the blood stream. This is in some disagreement with statements by others.

J. D. WILLEMS.

THE BEHAVIOR OF INTESTINAL PUTREFACTIVE PRODUCTS IN THE BLOOD AND URINE WHEN SHAKEN WITH CHARCOAL. E. BECHER, München. med. Wchnschr. **73**:1561, 1926.

Intestinal putrefactive products, including those in combination with sulphuric and glycuronic acids, are removed from the blood and urine by shaking with charcoal.

J. D. WILLEMS.

Microbiology and Parasitology

RELATIONSHIPS OF THE EPITHELIOMA CONTAGIOSUM VIRUS OF FOWLS TO THE VACCINE VIRUS. HOWARD B. ANDERVONT, Am. J. Hyg. **6**:719, 1926.

The results do not support the view that these viruses are identical.

THE ETIOLOGY OF HAVERHILL FEVER (ERYTHEMA ARTHRITICUM EPIDEMICUM). FREDERIC PARKER, JR., and N. PAUL HUDSON, Am. J. Path. **2**:357, 1926.

The evidence points to a bacterium as the cause. The name *Haverhillia multiformis* (new genus) is proposed as the name for this bacterium, which seems to belong to the order *Actinomycetales* (Buchanan). The source of the epidemic has not been found, but the serum of a cow in suspected herds contained agglutinins for the bacterium in question, which was obtained in the blood cultures of eleven of twenty-one patients examined. The characteristics of the bacterium are described.

DIFFERENTIAL CHARACTERISTICS OF THE AMOEBAE OF MAN IN CULTURE. J. H. ST. JOHN, Am. J. Trop. Med. **6**:319, 1926.

The medium consisted of egg and horse serum in Locke's solution. The details of structure and behavior that help to differentiate *Endamoeba histolytica* from other forms of amebas are described.

THE CHEMICAL STUDY OF BACTERIA XI. THE DEVELOPMENT OF A SYSTEMATIC ANALYTIC METHOD FOR THE COMPARATIVE STUDY OF BACTERIAL CELLS. T. B. JOHNSON, Am. Rev. Tuberc. **14**:164, 1926.

Johnson gives in this paper a programmatic outline of the chemical analysis of tubercle bacilli leading to purified chemical fractions, which will not only elucidate the structure of the micro-organism, but at the same time will furnish material for biologic research on chemically well defined bodies. The basic principles for the work are outlined and a table is given to explain the general plan of procedure. The essentials of the work are recorded in such a concise form that nothing short of a reprint of the article would give a fair representation of the author's views.

MAX PINNER.

TORULA MENINGITIS. FRANK B. LYNCH, JR., and EDWARD ROSE, *Ann. Clin. Med.* **4**:755, 1926.

Lynch and Rose feel that either the number of cases of torula meningitis in this country is increasing or else the disease is being recognized more frequently. There is no pathognomonic feature separating it from other sub-acute or chronic inflammatory conditions of the central nervous system, save the presence of *Torula* in the cerebrospinal fluid. All reported cases have terminated fatally.

WALTER M. SIMPSON.

THE INTESTINAL ORIGIN OF PERNICIOUS ANEMIA. KNUD FABER, *Ann. Clin. Med.* **4**:788, 1926.

The conviction that in some way or other pernicious anemia must be accounted for by abnormal conditions in the digestive organs, particularly in the intestinal canal, is growing. Intestinal atrophy plays no important part. The three principal arguments in favor of intestinal genesis of pernicious anemia are: First, pernicious anemia can be observed in a large number of cases in which *Bothriocephalus* is present in the intestinal canal, and it is usually promptly cured on expulsion of the worm. Second, pernicious anemia frequently occurs in patients with stricture of the small intestine. Third, anemia is often observed in patients with sprue. Faber found anemia in 36.5 per cent of 100 cases of chronic achylia gastrica, often quite mild, but severe in many. In some instances, the achylia was demonstrated many years before the appearance of the anemia. In achylia the stomach's disinfecting power is in abeyance. While the duodenum and upper jejunum are normally sterile or extremely poor in bacteria, one finds a rich and varied bacterial flora up into the duodenum in patients with achylia. Streptococci, *Bacillus coli* and many others are frequently encountered. In this deficient disinfection is an explanation of the periodic diarrhea so common in achylia, and it also makes possible the formation and absorption of hemolytic substances. The same explanation would hold for the blood picture of anemia seen in cancer of the stomach, which is usually accompanied by achylia.

Achylia gastrica is the most frequent, but not the only, cause of pernicious anemia. It may occur during pregnancy without achylia, probably as a result of the intoxication of pregnancy. It may develop during acute sepsis. Faber has reported five definite cases of pernicious anemia without achylia. A continually occurring intoxication is the most natural explanation, and in the majority of cases it is an intestinal intoxication.

WALTER M. SIMPSON.

E. HISTOLYTICA: LONGEVITY OF CYSTS IN VITRO. W. YORK and A. R. D. ADAMS, *Ann. Trop. Med.* **20**:317, 1926.

The eosin reaction may not be a reliable index of viability of endameba cysts; the cysts that stain with eosin are almost certainly always dead, but it is not so certain that those that do not stain with eosin are alive. A more reliable method of determining viability based on artificial cultures is described.

BACILLUS OZAENAE FOETIDAE, PEREZ AND BACILLUS PROTEUS IN OZAENA. ALEXANDER MICHAÏLOFF, *Bull. Johns Hopkins Hosp.* **39**:158, 1926.

It is concluded that the Perez bacillus is a member of the *Proteus* group. The frequency of the occurrence of *Proteus*-Perez bacilli in ozenae, and the

peculiar odor of the cultures, show that these organisms are concerned in the genesis of ozenae. Whether implanted primarily or secondarily, they are the cause of the fetor, the discharge and the ulceration.

NATIVE INFESTATION BY THE FISH TAPEWORM, *DIPHYLLOBOTHRIUM LATUM*: REPORT OF A CASE IN DETROIT. DAVID J. LEVY and MERLE PIERSON, J. A. M. A. **87**:848, 1926.

This is the tenth case from the Great Lakes region. It occurred in a Jewish girl born in Detroit.

ETIOLOGY OF GRANULOMA INGUINALE. J. A. McINTOSH, J. A. M. A. **87**:996, 1926.

The transmission is reported of inguinal granuloma by the subcutaneous implantation of a piece of granulation tissue from a spontaneously infected patient. The incubation period was forty-seven days. The Donovan body is regarded as the cause of inguinal granuloma and as unrelated to the Friedländer group of bacteria. Probably an actual break in the skin is necessary for inoculation. Fifteen spontaneous cases, all in negroes, are described.

ASPERGILLOSIS OF THE LUNGS AND ITS ASSOCIATION WITH TUBERCULOSIS. MARY E. LAPHAM, J. A. M. A. **87**:1031, 1926.

In the course of three years, ten cultures of *Aspergilli* were obtained from sputum sent in for the examination of tubercle bacilli. A plea is made for closer study of the occurrence of aspergillosis in man, as well as in cattle.

CREeping ERUPTION; REPORT OF FIRST CASE FROM MANITOBA. KRISTJAN J. AUSTMAN, J. A. M. A. **87**:1196, 1926.

* A case of creeping eruption caused by the first stage larva of the fly *Gastrophilus intestinalis* is described. Lombard's method of cleaning the epidermis with oil in order to observe the capillaries of the skin, was of help in locating and removing the larva which causes a red, papular, linear eruption over its pathway in the epidermis.

MUTATION FORMS OF TUBERCLE BACILLUS. H. C. SWEANY, J. A. M. A. **87**:1206, 1926.

In a study of various conditions under which mutation forms of the tubercle bacillus may develop, it is found that mutation takes place with regularity during vegetation, depending on the fundamental law of variation together with a particular alteration of environment.

A constant favorable environment is difficult to maintain for a long period of time either in old cultures or in lesions. In either instance, split forms may appear that are vastly different from the tubercle bacillus.

Accordingly, there are produced mutation forms that may assume a great variety of cultural, morphologic and pathologic characteristics.

The two most common types of mutants are the bacillary and the coccoid forms, although filter passing forms also appear to exist.

Bacillary forms may pass over into coccoid forms and granules, and coccoid forms may grow into bacilli if the medium is suitable.

The mutation forms appear to develop from some of the gram-positive granules, which are perhaps resting stages of tubercle bacilli. When cocci are formed, there is a tendency to develop first diplococci, and later various tetrads and staphylococcus-like forms may develop. Bacillary forms also may develop.

Many of these mutants are not pathogenic. Many are of low pathogenicity, while others undoubtedly play a definite rôle in disease processes.

The suggestion is offered that certain chronic diseases (like Hodgkin's disease and polyserositis) may be caused by mutation forms of tubercle bacilli.

AUTHOR'S SUMMARY.

UNDULANT FEVER FROM *BRUCELLA ABORTUS*; REPORT OF TWO CASES. C. M. CARPENTER and H. E. MERRIAM, J. A. M. A. **87**:1269, 1926.

Cultures like those of *Brucella abortus* were isolated from the blood of two men with undulant fever. In one of the patients the disease was much more severe than in the other and lasted for twelve weeks. In the case of this patient the organism was recovered from the blood seven times and from the urine twice. The agglutinin absorption test showed that the strains from these two patients were of the *abortus* rather than the *melitensis* variety. Abortions in heifers were produced by injecting the cultures intravenously. In guinea-pigs the cultures produced changes which in their gross appearance were like those produced by cultures of *Brucella abortus* isolated from cattle. Both the patients drank milk daily and freely and the milk consumed by the patient who was most severely ill contained *Brucella abortus* in large numbers. The possibility of infection from swine or from goats seems to be excluded in these two cases, and cow's milk evidently is the most likely source of infection.

BACTERIOPHAGY IN URINARY INFECTION: I. THE INCIDENCE OF BACTERIOPHAGE AND OF *B. COLI* SUSCEPTIBLE TO DISSOLUTION BY THE BACTERIOPHAGE IN URINES; PRESENTATION OF CASES OF RENAL INFECTION IN WHICH BACTERIOPHAGE WAS USED THERAPEUTICALLY. N. W. LARKUM, J. Bact. **12**:203, 1926.
II. BACTERIOPHAGY IN THE BLADDER. Ibid. **12**:225, 1926.

Bacteriophage and *Bacillus coli* susceptible to its action are found in about 25 per cent of the urines from patients having urinary infections. Normal, bacteria-free urines do not contain bacteriophage. Bacteriophage was found exclusively in the urines from persons having acute infections. Four patients subjected to treatment with bacteriophage showed definite improvement after the treatment.

Infection of the bladder with lysogenic strains of *B. coli* is alone responsible for the existence of bacteriophage in the urine. Lysis of colon bacilli can take place in the bladder through the action of bacteriophage, but is modified by concomitant actions of the urine, mucous and bladder tissues.

ARTHUR LOCKE.

THE CHEMICAL STUDY OF BACTERIA: XII. THE ALBUMIN-GLOBULIN FRACTION OF THE TUBERCLE BACILLUS. R. D. COGHILL, J. Biol. Chem. **70**:439, 1926.
XIII. THE ALKALI-SOLUBLE PROTEIN OF THE TUBERCLE BACILLUS. Ibid. **70**:449, 1926.

A small amount of a basic protein, devoid of complement-fixation capacity, may be extracted from dried, defatted and pulverized tubercle bacilli by treatment with water or 5 per cent salt solution, followed by adequate centrifugalization. It may be precipitated from solution with acetic acid and gives the usual protein reactions, including the Molisch test, although not giving any other tests for sugar either before or after hydrolysis. It is not stated whether this preparation, adequately centrifugalized from suspensions of bacterial protoplasm, has any tuberculin activity.

Prolonged extraction with tenth normal sodium hydroxide, during which the suspension may turn dark and an odor of ammonia become evident, yields a protein preparation less basic than the former and practically devoid of tuberculin reaction.

ARTHUR LOCKE.

COMPARATIVE STUDIES OF HERPETOMONADS AND LEISHMANIAS. CULTIVATION OF HERPETOMONADS FROM INSECTS AND PLANTS. HIDEYO NOGUCHI and EVELYN B. TILDEN, J. Exper. Med. **44**:307, 1926.

DIFFERENTIATION OF THE ORGANISMS BY SEROLOGICAL REACTIONS AND FERMENTATION TESTS. HIDEYO NOGUCHI, Ibid., p. 327.

Morphologic differentiation of the flagellates studied, while not impossible, is subject to error by reason of the variations due to age and cultural conditions. The flagellates of the latex-feeding insects, the plants, the flies and the mosquitoes can readily be distinguished from *Leishmania* by their rapid growth at 37 C., but their differentiation from one another is possible only by serologic and fermentation reactions.

The data presented suggest that the biologic characteristics of flagellates of the *Herpetomonas* group may be utilized with advantage for identification of a species which occurs in different environments and for separation of different species when they are found in the same environment. If the leishmania parasites pass the flagellated or herpetomonad stage of their life history in some invertebrate host, it may be possible by tests of the kind described to distinguish them from the nonpathogenic herpetomonads which are so widely distributed among insects and plants.

BIOLOGY OF BACTERIUM LEPISEPTICUM: PHYSICAL, CULTURAL AND GROWTH CHARACTERISTICS OF DIFFUSE AND MUCOID TYPES AND THEIR VARIANTS. LESLIE T. WEBSTER and CAPAR G. BURN, J. Exper. Med. **44**:343, 1926.

VIRULENCE OF DIFFUSE AND MUCOID TYPES AND THEIR VARIANTS. Ibid., p. 539.

A "D" type and a "mucoid" type of *Pateurella* organisms have been recovered from rabbits; the former is relatively rare, is virulent and not vegetative; the latter is common, less virulent and readily adaptable to a vegetative existence. The virulence of the different strains of each type appears to be about the same and not to be affected by passage through animals or nontoxic, nutrient mediums. Although each possesses distinctive and constant characteristics, the authors feel that the possibility of mutual relationship has not been entirely excluded. Investigation of bacterial properties associated with virulence has as yet given no definite and positive results.

SUSCEPTIBILITY OF MICE TO INOCULATION WITH TUBERCLE BACILLI. C. H. BROWNING and R. GULBRANSEN, J. Hyg. **25**:323, 1926.

Mice are susceptible to intraperitoneal inoculation of human and bovine tubercle bacilli in doses of 0.75 mg. of moist culture. With a fairly virulent bovine type, 0.005 mg. caused infection about as well as the larger doses. The bovine strains seemed to act more rapidly than the human.

APPARENT MUTATION OF STREPTOCOCCI FROM ACUTE MALIGNANT ENDOCARDITIS. KATHARINE M. HOWELL and DOROTHY A. BEVERLEY, J. Infect. Dis. **39**:12, 1926.

From the blood of a case which was clinically one of acute malignant endocarditis there were isolated on three successive occasions two bacteriologically

distinct organisms, *Streptococcus hemolyticus* and *Streptococcus viridans*. The two strains were morphologically different; the morphologic differences have remained constant over a period of two years. The sugar reactions, which were also different, have remained constant for the same period.

The hemolytic streptococcus strain seven months after isolation lost its hemolyzing quality and remained constantly thereafter anhemolytic. One constant mutation, therefore, occurred.

Brief animal passage indicated that the two strains were different. It was impossible by this experimental procedure to split off a viridans variant from the hemolytic streptococcus or its anhemolytic form, or the reverse. Transient variants occasionally occurred.

The immunologic reactions, although variable, suggested that there was only a single strain, the hemolytic streptococcus. It is possible that the parasitic growth of two closely related bacterial strains may so alter their immunologic reactions that differentiation by such reactions becomes impossible.

Protection experiments with immune serums indicated that the two strains were different.

AUTHORS' SUMMARY.

DIFFERENCES IN PEROXIDE PRODUCTION AND METHEMOGLOBIN FORMATION OF GREEN (ALPHA) STREPTOCOCCI. EUGENIA VALENTINE, J. Infect. Dis. 39:29, 1926.

Green (alpha) streptococci may be differentiated into two varieties based on peroxide production and methemoglobin formation of from eighteen to twenty-four hour broth cultures. The *X* variety gives a positive peroxide reaction with benzidine and a strong methemoglobin reaction when added to washed sheep red blood cells in the test tube; the *Y* variety at that period is negative for peroxide and oxidizes hemoglobin to methemoglobin only after the addition of sterile broth, serum, etc.

Four hour cultures of the two varieties are found to be alike in peroxide and methemoglobin production but after an incubation of from eighteen to twenty-four hours at 37 C. a difference in action is apparent.

The growth activity of the two varieties is shown to be different. The *X* strains reach the height of the growth curve after from twelve to fourteen hours' incubation, while active multiplication of the *Y* strains drops decidedly after six hours.

Difference in the methemoglobin reaction of the *X* and *Y* strains is evident when salt solution suspensions of washed organisms are tested. To complete the reaction of the salt suspensions of *X* cultures only a small amount of accessory or oxidizable substance is required, while the *Y* salt suspensions are active only in the presence of a highly oxidizable or growth stimulating substance. Sterile filtrates of *X* or *Y* broth cultures complete the methemoglobin reaction of *X* cocci suspended in salt solution but fail to activate the *Y* cocci.

The *X* streptococci more closely approach pneumococci in their peroxide-producing and methemoglobin-forming properties.

Certain possible correlations with the work on the oxidation-reduction systems of the pneumococcus are discussed.

AUTHOR'S SUMMARY.

RÔLE OF BACTERIOPHAGE IN STREPTOCOCCUS INFECTIONS. L. O. DUTTON, J. Infect. Dis. 39:48, 1926.

Streptococci react in a characteristic manner when mixed with bacteriophage, some being resistant, others sensitive. This variability results in colony forms

that are characteristic as well as in variation in regard to transmissible lysis. On the basis of such data the strains studied are divided into six types: Type 1—strains give growth on the isolation culture, colony form irregular with spontaneous lysis, no growth in subsequent transplants. Type 2—strains are essentially like type 1 except that transplants on agar grow, reproducing the characteristic colonies, while transplants in broth remain sterile. Type 3—strains have normal colonies, normal growth in all transplants, and are susceptible to the action of a lytic principle. Type 4—strains are like type 3, except that they are not susceptible to transmissible lysis. Type 5—strains have a characteristic sensitive type colony which is rough, and eroded; showing the phenomenon of transmissible lysis. Type 6—strains show resistant colonies which are rough and irregular, with small subcolonies developing in the original after a varying period of incubation; no transmissible lysis.

A possible explanation is that most strains of streptococci are mixed with bacteriophage, and that the colony forms are the result of the reaction of the bacterium to the phage, this ranging from complete sensitiveness to complete resistance.

Study of the distribution of the various types in streptococcus blood infections indicates that in those patients who recover, the strain is sensitive to bacteriophage and mixed with it. In cases that are fatal the strains isolated are resistant, and mostly unmixed with bacteriophage.

Studies of the normal flora of the throat indicate that the source of the bacteriophage in streptococcus blood infections probably is the normal throat streptococci, many strains of which are mixed with bacteriophage.

AUTHOR'S SUMMARY.

INVESTIGATIONS ON ROCKY MOUNTAIN SPOTTED FEVER IN COLORADO. FREDERICK E. BECKER, J. Infect. Dis. 39:81, 1926.

Rocky Mountain spotted fever exists in Colorado with an average annual incidence of more than 2.57 cases and a mortality of 1.5 cases. The mortality rate lies between that of Idaho and the Bitter Root Valley in Montana.

The disease is widely spread throughout the mountainous regions of the state with certain centers more heavily infected than others.

The disease in several patients and in guinea-pigs infected from these patients and from wood ticks differed from the typical Montana form in the exhibition of marked gastro-intestinal symptoms and lesions.

Many micro-organisms, other than *Dermacentor rickettsii*, are found in local ticks. That these have pathogenic properties has not been demonstrated, but further work seems indicated.

Certain symptoms of malaise, not typical of Rocky Mountain spotted fever, frequently follow tick bites in this state. Evidence supports the view that they are due to the tick bite, but are not a part of the clinical picture of Rocky Mountain spotted fever.

AUTHOR'S SUMMARY.

THE ACTIVITY OF AN ANTICOLON BACTERIOPHAGE IN SYNTHETIC MEDIUM. JANET ANDERSON CALDWELL, J. Infect. Dis. 39:122, 1926.

An anticolon bacteriophage was transmitted by serial passage in a nonprotein medium. Enhancement of virulence toward the organism used occurred during the passage. Virulence toward a different organism was not decreased. Titra-

tions of anticolon filtrates were more satisfactory in synthetic medium than in broth. The active filtrates did not give the biuret reaction and did not produce anaphylaxis in guinea-pigs.

AUTHOR'S SUMMARY.

GAS PRODUCTION BY BACTERIAL SYNERGISM. W. L. HOLMAN and D. M. MEEKISON, *J. Infect. Dis.* **39**:145, 1926.

Synergism is a useful term for the cooperative phenomena discussed in this paper.

One of the pair of bacteria must be capable of splitting the test substance and forming acid. The other must be able to form gas from monosaccharides.

The stage of growth and functional activity of the bacteria play important rôles in the phenomena.

The hydrogen ion concentration and its regulation by the bacterial products are important factors in obtaining various results.

Gas production can be inhibited as well as stimulated and the two phenomena are closely related through the various factors affecting the metabolism of bacteria.

All grades of these phenomena can be obtained, depending on the characters of the bacteria used, their age and the use of different mediums.

The gas analysis shows a different gas ratio for the pair from that obtained with the gas producer alone.

There is good evidence that the acid and gas-producing functions are different in time and probably depend on different endo-enzymes.

There is a wide field for the biochemist and physicist to explore to broaden our meager knowledge of the intimate metabolism of bacteria.

AUTHORS' SUMMARY.

STUDIES ON BOTULINUM TOXIN IN THE ALIMENTARY TRACT OF HOGS, RABBITS, GUINEA-PIGS AND MICE. G. M. DACK and J. GIBBARD, *J. Infect. Dis.* **39**:173, 1926.

There was no evidence that type A botulinum toxin was absorbed by the intestinal contents in vitro even when the p_H was shifted with three thousandth normal hydrochloric acid or disodium acid phosphate.

Hogs were found to be very resistant to large oral doses of toxin; in some cases as much as ten million minimum lethal doses for mice were fed without producing any ill effects. Toxin was not demonstrated in 1 cc. of blood taken from a hog which had been fed nine million (mouse) minimum lethal doses.

Toxin was not demonstrated in the blood of rabbits ninety minutes after the feeding of botulinum toxin.

Two rabbits received intravenous injections of small graded doses of toxin. Toxin was demonstrated in the blood stream eighteen hours later.

Two rabbits receiving large doses of toxin in the small intestine showed symptoms of botulism within seven days. One died on the seventh and the other on the fourteenth day. Toxin was not demonstrated in 0.5 cc. of blood taken from each of these animals after ninety minutes or in 1 cc. taken after eighteen hours.

Two rabbits received intracecal injections of toxin. One of the rabbits remained normal. The other died the following day and toxin was found in the blood.

Toxin could not be demonstrated after ninety minutes in the blood of guinea-pigs which had received injections of toxin in the ligated stomach but was

demonstrated in the serum from the heart blood taken after death. Toxin was not demonstrated in the blood of animals receiving injections of toxin in the ligated small intestines or ligated cecum, either after ninety minutes or in the serum from the heart blood taken after death. There was one exception in a guinea-pig which had received an intracecal injection, the cecum of this animal being ruptured.

Two guinea-pigs were given intracecal injections of toxin; one died seventeen days later with typical symptoms of botulism; the other remained normal.

Mice fed botulinum toxin failed to develop any symptoms of botulism. Toxin was demonstrated in the stomach and small intestines of mice three hours after they had been fed toxin but not twelve hours later.

The evidence obtained from these experiments indicates that, if an animal susceptible to oral administration of toxin has been fed toxin, in addition to the toxin absorbed from the stomach and small intestines, there is perhaps some continued absorption from the cecum, although the amount is probably slight. Further studies are being made on the absorption of toxin from the cecum.

AUTHORS' SUMMARY.

PERMEABILITY OF THE SMALL INTESTINE OF RABBITS AND HOGS TO BOTULINUM TOXIN. G. M. DACK and J. GIBBARD, *J. Infect. Dis.* **39**:181, 1926.

A loop of small intestine in each of six rabbits was injected with botulinum toxin and perfused with blood from the same animal for intervals varying from thirty minutes to two hours and twenty minutes. Small quantities of toxin were demonstrated in the perfused blood, and often only symptoms of botulism were produced in the mice receiving 0.5 cc. quantities of the undiluted blood or serum. Never was any toxin demonstrated in the 1:10 dilution.

Two hogs were treated in a similar manner with the result that toxin was demonstrated in one case and not in the other. Only a small amount of toxin was found in the one case; a mouse receiving 0.5 cc. of serum taken at an hour and a half interval died in four days.

The toxin introduced into the ligated loop of intestine showed little if any decrease in potency during the course of the experiment.

AUTHORS' SUMMARY.

BACTERIOLOGIC STUDIES IN ENDOCARDITIS. WILLIAM A. KREIDLER, *J. Infect. Dis.* **39**:186, 1926.

In a relatively high percentage of cases of endocarditis a streptococcus can be isolated from the blood, and also frequently from vegetations on heart valves and infarcts in cases coming to necropsy. In the cases studied, all but one yielded an organism producing green colonies on blood agar. In the one exception a typical beta hemolyzer was isolated. None of the strains liquefied gelatin. All strains produced acid in litmus milk. The fermentation of carbohydrates was found to be irregular. No satisfactory classification can be made on this basis. The streptococci were insoluble in bile, and of low virulence. Cultivation of organisms in collodion sacs in the peritoneal cavity of rabbits did not change their morphologic and cultural characteristics, or their serologic reactions.

No cross agglutination, cross precipitation or cross complement fixation occurred among the strains isolated. From these observations and the fact that no absorption of agglutinins took place, it appears impossible to group these streptococci on the basis of their serologic reactions. Immune bodies for the homologous strain were found in the blood of all patients tested. Lesions of

the heart valves similar to those occurring in human beings can be produced experimentally in animals by the injection of these organisms directly into the heart and intravenously. Apparently, no exotoxin, endotoxin or poisonous split protein could be liberated from the strains studied. When the foregoing facts are considered, there seems to be ground for the belief that endocarditis with its associated lesions is produced by streptococci, usually members of the viridans group. This statement is based on the fact that inoculation of rabbits with cultures of the groups studied has led to the development of valvular lesions comparable to those seen in human beings.

AUTHOR'S SUMMARY.

CULTURE MEDIUM FOR DIFFERENTIATING ORGANISMS OF THE TYPHOID-COLON-AEROGENES GROUPS AND FOR THE ISOLATION OF CERTAIN FUNGI. JAMES S. SIMMONS, J. Infect. Dis. 39:209, 1926.

A solid citrate agar medium has been prepared by adding agar and bromthymol blue to one of the citrate solutions recommended by Koser.

Escherichia coli was markedly or completely inhibited on this citrate agar and did not change the color of the medium; while *Aerobacter aerogenes* grew luxuriantly, forming large raised greenish-blue colonies and producing blue in the medium. The results in every test were comparable to those obtained with the fluid citrate medium.

Eberthella typhi, *Eberthella dysenteriae*, *Eberthella paradysenteriae*, *Salmonella paratyphi*, *Salmonella pullora* and *Salmonella morgani* failed to grow or change the color of citrate agar; while *Salmonella schotmülleri*, *Salmonella aertrycke*, *Salmonella enteritidis* and *Salmonella typhi-murium* grew luxuriantly forming translucent greenish-blue colonies and changing the color of the medium to a deep Prussian blue.

A number of unrelated organisms, including *Escherichia coli*, and a number of pyogenic cocci, etc., failed to grow on citrate agar.

Certain fungi and fungi imperfecti, including a number of sacchyromyces, twenty strains of *Monilia*, two of *Sporotrichum schenki* and one of *Madurella mycetoma*, grew luxuriantly on citrate agar forming yellow or yellowish-orange colonies and changing the color of the medium to a deep yellow or orange.

AUTHOR'S SUMMARY.

A COMPARISON OF STRAINS OF BRUCELLA ABORTUS ISOLATED FROM MAN WITH THOSE FROM CATTLE. C. M. CARPENTER, J. Infect. Dis. 39:215, 1926.

The first generation of cultures of *Brucella abortus* isolated from man was difficult to cultivate, but the second and following generations have grown well on nutrient agar in unsealed tubes.

The human strains have proved highly virulent for guinea-pigs but not more virulent than certain bovine strains with which the author has worked. Four of the strains have produced lesions in guinea-pigs which could not be distinguished from lesions produced by virulent types isolated from milk, placenta and bovine fetus.

Abortion was produced in five pregnant heifers by the intravenous injection respectively of five of the cultures. The organism was recovered from the colostral milk, placenta and fetus. The infection established itself in the udder and was present in the milk of one case for six and one-half months after the animal aborted. The cultures isolated from man were more toxic for the pregnant heifers than the bovine types and produced abortion in a shorter time than bovine strains reported by other observers.

AUTHOR'S SUMMARY.

AGGLUTININS FOR *BRUCELLA ABORTUS* IN THE BLOOD OF MAN. C. M. CARPENTER, J. Infect. Dis. **39**:220, 1926.

The serums from four of twenty patients showing symptoms suggestive of undulant fever contained specific agglutinins for *Brucella abortus*. The serum from a fifth patient who showed typical symptoms of recurrent or undulant fever agglutinated the abortus antigen completely in a dilution of 1:120. Sufficient serum could not be obtained for an agglutinin absorption test.

Brucella abortus was isolated from the blood of five of the twenty patients. In the blood serums of two of these no abortus agglutinins could be demonstrated. However, one serum contained agglutinins for typhoid bacilli while the other gave a four plus Wassermann reaction.

The titer of the serums varied from 1:30 to 1:480.

In the light of these observations it is evident that the agglutination test cannot be relied on to detect the presence of *Brucella abortus* in man.

AUTHOR'S SUMMARY.

INFLUENCE OF HIGH PARTIAL PRESSURES OF OXYGEN ON THE GROWTH OF CERTAIN MOLDS. HOWARD T. KARSNER and OTTO SAPHIR, J. Infect. Dis. **39**:231, 1926.

Concentrations of oxygen of 76 per cent and more, exercise a definite inhibitory effect on certain molds. In a general way this is most apparent in molds with the capacity for parasitism. It is not a fixed corollary of parasitism, because some of the parasitic molds, more especially the plant parasites, show no inhibition.

There is no fixed relation between the degree of inhibition and the percentage of oxygen concentration, although the higher percentages appear to be more certain in effect. The inhibitory effect is apparent from the time growth of the colony starts until the end of the observation. In no instance was the organism killed by the oxygen percentages employed and on removal from the oxygen chamber growth appeared to progress at a normal rate.

The production of pigment by the molds is not altered by growth in any of the oxygen concentrations used.

AUTHORS' SUMMARY.

DRUG FASTNESS IN ITS RELATION TO THE RESISTANCE OF CERTAIN ORGANISMS TOWARD FAMILIAR GERMICIDES. PERCY D. MEADER and WILLIAM A. FEIRER, J. Infect. Dis. **39**:237, 1926.

Definite drug fastness has been developed in one strain of *Bacillus typhosus*, three strains of *Bacillus coli* and one strain of *Bacillus lactis aerogenes* to silver nitrate, mercurochrome, formaldehyde, acriflavin, hexylresorcinol and phenol.

The highest degree of drug fastness was developed in cultures exposed to the action of germicides containing a heavy metal (silver nitrate and mercurochrome).

When highly developed the drug fast character is specific.

Drug fast strains are, in general, more susceptible to the action of formaldehyde, silver nitrate and mercurochrome than were the original strains on isolation. The majority of drug fast strains do not show increased susceptibility to acriflavin, hexylresorcinol and phenol.

None of the drug fast strains retains its ability to grow on the final maximum concentration of the germicide after cultivation on drug-free agar for five days.

All of the organisms of the coli-typhoid group studied in this investigation exhibit wide variations in their behavior toward the various germicides employed.

AUTHORS' SUMMARY.

STUDIES ON THE ETIOLOGY OF EPIDEMIC ENCEPHALITIS: I. THE STREPTOCOCCUS.
ALICE C. EVANS and WALKER FREEMAN, Pub. Health Rep. 41:1095, 1926.

A pleomorphic streptococcus, highly virulent for rabbits when inoculated intracerebrally, was obtained from the nasal washings, heart blood, and mesencephalon of a patient with epidemic encephalitis.

So far as the comparative tests have been made, this streptococcus agreed with the streptococci obtained from cases of epidemic encephalitis by Von Wiesner and by Rosenow. Apparently several other investigators have cultivated the same organism in their studies of the disease.

When inoculated intravenously into rabbits the streptococcus shows a tendency to elective localization in the brain.

In rabbits and in monkeys it produces nervous symptoms which in some cases simulate the disease in man.

Rabbits inoculated with this streptococcus do not show inflammatory lesions outside of the central nervous system. The meninges are heavily infiltrated with lymphocytes and leukocytes, the inflammation spreads to the cerebral substance by direct extension and along the small vessels, penetrating into the brain. There are severe parenchymatous degenerative changes in the nervous tissue and reaction of the neuroglia. The sheaths of the blood vessels are found infiltrated by lymphocytes. The reaction is sometimes most marked in the mesencephalon.

In monkeys a greater tendency toward leukocytic reaction is noted and in two instances large areas of hemorrhagic inflammation in the basal ganglia were noted.

AUTHOR'S SUMMARY.

NEW SPECIES OF MONILIA PATHOGENIC FOR MAN. FREDERICK W. SHAW, Science 64:300, 1926.

The organism was isolated from small, white granules in the sputum from a case of supposed pulmonary tuberculosis. There were no tubercle bacilli in the sputum. The organism, for which the name *Monilia richmondi* is proposed, grows as budding cells on dextrose agar and produces budding forms as well as branching threads in dextrose broth. It is pathogenic for rabbits, guinea-pigs and white rats.

A DISEASE OF RABBITS CHARACTERIZED BY A LARGE MONONUCLEAR LEUCOCYTOSIS, CAUSED BY A HITHERTO UNDESCRIBED BACILLUS BACTERIUM MONOCYTOGENES (N. SP.). E. G. D. MURRAY, R. A. WEBB and M. B. R. SWANN, J. Path. & Bact. 29:407, 1926.

In investigating a spontaneous epidemic disease of rabbits, a micro-organism was isolated in pure cultures which reproduced the characteristic lesions of the natural disease.

The bacteriologic characters of this bacillus are described and the impossibility of identifying it with previously recorded organisms justifies its being considered a new species. The name *Bacterium monocytogenes* is proposed.

Animal passage raised "virulence" when the doses were well chosen, and increased virulence accentuated the production of necrotic lesions. Overwhelming doses of culture resulted in lowering of "virulence" by animal passage.

Bacterium monocytogenes, in doses less than the minimum lethal dose, produced in the circulating blood of rabbits an extreme monocytosis. The responses of the other white cells were either transient or inconstant.

Repeated doses of the organism became progressively less effective as stimuli to large mononuclear production.

The cell content of the thoracic duct did not reflect the high degree of monocytosis in the circulating blood.

On intrapleural injection of peptone broth and *Bacillus coli*, the cells of the resultant exudate were primarily polymorphonuclears, even though the circulating blood showed a high monocytosis. With intrapleural injection of *Bacterium monocytogenes*, when the blood stream was rich in large mononuclears, a pleural exudate containing 30 per cent of these cells was obtained.

Phagocytosis experiments in vitro showed that the large mononuclears, while they phagocytosed *B. coli* indifferently, took up *Bact. monocytogenes* with avidity in all respects equal to that of the polymorphonuclear neutrophils.

AUTHOR'S SUMMARY.

REPORTS FROM THE ROYAL SOCIETY'S KALAR AZAR COMMISSION IN CHINA: I. SEROLOGICAL STUDIES ON CHINESE KALA AZAR. E. HINDLE, P. C. HOU and W. S. PATTON, Proc. Roy. Soc. series B, **100**:368, 1926. IV. RESISTANCE OF LEISHMANIA CULTURES TO COLD. HINDLE and PATTON, *ibid.* **100**:385, 1926.

A complement fixation test may be used, within limitations, for the diagnosis of kala-azar. A suspension of the spleen of a heavily infected animal is used as antigen.

Leishmania, in the flagellate stage, are able to withstand intermittent exposure to temperatures of -12°C . for a period of at least ten days and may remain active after a freezing and thawing procedure.

ARTHUR LOCKE.

THE ACTION OF EUCALYPTOL ON THE TUBERCLE BACILLUS IN VITRO. PAUL FABRY, Ann. de l'Inst. Pasteur **40**:521, 1926.

A culture medium containing 0.2 per cent eucalyptol kills a culture of bovine tubercle bacilli in two months. A culture of the same organism is killed by contact with eucalyptol vapors at 37°C . for thirty-six days. The vapors also inhibit all development of the organism. A guinea-pig inoculated subcutaneously with 1 mg. of tubercle bacilli was kept in an atmosphere with eucalyptol vapor for twenty-nine days. It increased in weight and after two months and six days was living and healthy.

G. B. RHODES.

BACTERIOLOGIC STUDIES OF EPIZOOTIC ABORTION IN MARES. L. PANISSET and L. VERGE, Ann. de l'Inst. Pasteur **40**:524, 1926.

Several organisms were isolated from the fetus, membranes and fluids. Streptococci, gram-positive, oval or diplococci, in chains of from five to forty, isolated from three cases were found to be identical. They were not hemolytic and did not ferment mannite and so differed from the streptococcus from horses with thumps, in which serum also did not produce agglutination. A gram-negative, aero-anaerobic organism was judged to be an atypical colon bacillus. *Bacillus abortus-equi*, a coccobacillus, gram-negative, motile, evidently of the group *Salmonella*-closely related and possibly identical with *Bacillus enteritidis* Breslau (or *Bacillus aertrycke*), was agglutinated by serum from mares that had aborted, and provoked abortion in guinea-pigs, and immunity in rabbits. Therefore, hopes are expressed for the successful vaccination of mares.

E. B. PERRY.

SOME USEFUL MODIFICATIONS OF MY CULTURE MEDIUM AND OF THE TECHNIC OF ISOLATING KOCH'S BACILLUS IN PURE CULTURE FROM EXCRETIONS AND OTHER TUBERCULOUS MATERIAL. G. PETRAGNANI, *Boll. d'Ist. Sieroterap.* Milan. 5:173, 1926.

The details of preparing a medium containing milk, potato, peptone, eggs, glycerol and malachite green are given. From centrifugalized specimens of serous exudates, urine or other fluids a portion of the sediment is mixed with an equal amount of a 4 per cent solution of sodium hydroxide, incubated in a water bath for an hour or more, diluted with sterile tenth normal salt solution (specific gravity 1.20) to obtain the correct reaction, and finally cultured in amounts varying from 0.2 to 1.0 cc., the tubes being sealed with paraffin. Further details are given.

AUTHOR'S SUMMARY.

RELATIONS BETWEEN THE VIRUSES OF EPIDEMIC ENCEPHALITIS, ACUTE ANTERIOR POLIOMYELITIS AND HERPES. M. GERBASI and M. GIUFFRÉ, *Riv. di patol. sper.* 1:94, 1926.

Material and cultures from ten cases of labial herpes, five children with epidemic encephalitis and three children with acute anterior poliomyelitis were used in cross inoculations and tests of various kinds. The experimental encephalitis induced in rabbits by each of these three viruses seemed to be identical in all, both in symptoms and pathologic anatomy. The meningeal and encephalitic reaction to these filtrable viruses seems to be identical further with the encephalitis produced by inoculation with the virus of contagious epithelioma of fowls and of infectious encephalitis in horses, and also the spontaneous encephalitis of rabbits. Deviation of complement tests were positive for each disease and reciprocally positive with epidemic encephalitis and acute poliomyelitis, but not with herpes. All the data presented confirm the close kinship or even identity of the viruses of the two former. The special lesions were punctate hemorrhages in the white matter of the brain and keratitis from inoculation of the cornea; there were no foci of necrosis in the white or gray matter.

E. P. CUSHING.

KALA-AZAR IN CHILDREN IN MADRID. J. BRAVO Y FRIAS, *Arch. españ. de pediat.* 10:423, 1926.

About 300 cases of this form of leishmaniasis have been published in Spain. In three recent cases, the disease was in an acute febrile form and the children, aged 6, 11 and 12, all died. The diagnosis was confirmed by puncture of the much enlarged spleen. Contact with dogs or other domestic animals could not be demonstrated for the majority of the cases. The endemic seems to be spreading, and it is probable that in many cases classified as malaria in the past, the splenomegaly was due to leishmaniasis.

FILTRATION EXPERIMENTS WITH TUBERCLE BACILLI. ALFRED FESSLER, *Central f. Bakteriöl.* 98:148, 1926.

The claim by Vaudremer and others that the tubercle bacillus may occur in filtrable form is not supported.

MORPHOLOGY AND BIOLOGY OF ENTEROCOCCI. HERTHA SCHÖNFELD, *Centralbl. f. Bakteriol.* **99**:388, 1926.

DIFFERENTIATION OF ENTEROCOCCUS FROM STREPTOCOCCUS VIRIDANS AND THEIR RELATION TO STREPTOCOCCUS LACTIS. KURT MEYER and HERTHA SCHÖNFELD, *Ibid.*, p. 402.

HEMOLYTIC ENTEROCOCCI. KURT MEYER, *Ibid.*, p. 417.

SPECIFIC AGGLUTINATION OF ENTEROCOCCI. KURT MEYER and HANS LÖWENSTEIN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **47**:39, 1926.

The enterococci occur mostly as oval diplococci; the colonies on blood agar are colorless or grayish white, rarely hemolytic. They split esculin and grow in 20 per cent bile in peptone water. They consequently form a group different from the viridans and hemolytic groups of streptococci.

The enterococci are serologically distinct: agglutinating serum against enterococci has no effect on streptococci of the hemolytic and viridans groups, either directly or in absorption tests. There are at least three different serologic groups among enterococci.

CLINICAL AND BACTERIOLOGIC EXPERIENCES WITH ENDOCARDITIS LENTA. W. LEHMAN, *Klin. Wchnschr.* **5**:1408, 1926.

It is sufficiently established that endocarditis lenta is due to an infection with streptococcus viridans, and the clinical picture of this disease is so typical, that the designation endocarditis lenta must be restricted to instances in which the symptoms are characteristic and the viridans etiology is assured.

ARTHUR LOCKE.

THE PRODUCTION OF CHOLERA TOXIN. M. HAHN and J. HIRSCH, *Klin. Wchnschr.* **5**:1569, 1926.

Maximal multiplication of the cholera vibrio is obtained in from six to ten hours by the fractional addition of small quantities of dextrose and simultaneous maintenance of the reaction at P_H 8.0. Centrifugating mediums, sterilized by chloroform or toluene, and injected intraperitoneally in doses of from 0.25 to 1 cc. kill a guinea-pig in from twelve to eighteen hours with a sudden fall of temperature, respiratory spasms and paralysis. Post mortem there are a peritoneal exudate, reddening and enlargement of the suprarenals and marked hyperemia of the small bowel. Cultures are sterile. Berkefeld filtration greatly weakens, and heating to 70 degrees for thirty minutes destroys the toxic action. Guinea-pigs receiving a sublethal dose can withstand a double lethal dose in seven days.

J. D. WILLEMS.

EXPERIMENTAL INVESTIGATIONS ON THE PATHOLOGY AND THERAPY OF SPIROCHETAL DISEASES: IV. THE BEHAVIOR OF THE RELAPSING FEVER SPIROCHETE IN THE SKIN OF NORMAL AND IMMUNIZED ANIMALS. G. STEINER, HENNING and STEINFELD, *Klin. Wchnschr.* **5**:1599, 1926.

Normal rats were infected with relapsing fever by removing a small piece of skin, injecting it with the organisms and replacing it. Dark-field examination demonstrated the spirochetes in the blood stream after from four to five days. The same developed when a fragment of infected liver was placed with-

in a small sack formed from freshly removed normal skin, and this introduced subcutaneously into a normal rat. When the skin of immunized rats was used, infection did not follow. The spirochetes penetrated the normal skin directly, but not that of an immunized animal.

J. D. WILLEMS.

THE GERMICIDAL EFFECTS OF X-RAYS. W. SCHEPMANN and H. FLECKE, Klin. Wchnschr. 5:1608, 1926.

Streak inoculations of various bacteria were exposed to different wave lengths of the roentgen rays. The larger rays were most effective.

J. D. WILLEMS.

INCUBATION TIME. F. HAMBURGER, München. med. Wchnschr. 73:1233, 1926.

The incubation time is known quite accurately for the obligative infections and is fairly constant. The time required for the development of an allergic condition after a facultative infection is likewise fairly constant. Diphtheria and influenza have no true incubation time.

ARTHUR LOCKE.

THE SPECIAL POSITION OF THE NERVOUS SYSTEM TO SPIROCHETAL INFECTION. F. PLAUT, München. med. Wchnschr. 73:1552, 1926.

When rabbits are injected suboccipitally with the spirochetes of relapsing fever an infection follows which may be limited to the nervous system and produce meningitis. The spirochetes can be found in the spinal fluid, seldom in the blood stream. Rats are infected by an emulsion of the brain of these rabbits—not by the liver, spleen, blood or spinal fluid. When the rabbits are injected intraperitoneally with the spirochetes, infection of the nervous system alone follows. The conclusion is that the spirochete of relapsing fever has a special affinity for the nervous system of the rabbit, analogous to that of the *Spirocheta pallida* and human syphilis.

J. D. WILLEMS.

METALLIC SALTS IN THERAPY. L. E. WALBUM, Ztschr. f. Immunitätsforsch. u. Exper. Therap. 47:213, 1926.

In a large series of experiments the following metals appeared to hinder the development of tuberculosis in infected guinea-pigs: barium, aluminum, lanthanum, cerium, selenium, cadmium, molybdenum and ruthenium.

THE PROPAGATION OF TUBERCULOSIS IN THE BODY. H. HUEBSCHMANN, Ztschr. f. Tuberk. 45:177, 1926.

"The primary complex" as an anatomic entity described by Ranke indicates the site of the primary infection. About 84 per cent of all primary foci are found in the lung (according to the author's own and Ghon's material). In the intestines are found about 14 per cent of the primary foci. Infection of those organs which have no direct connection with the outside is always carried by the blood stream. As to pulmonary reinfection leading to "tertiary phthisis," Huebschmann believes that endogenous and exogenous propagation occurs, that the typical apical lesions are usually hematogenous-endogenous and that other localizations in the lung are mostly exogenous. In the further propagation the hematogenous way is more important than the descending canalicular way.

MAX PINNER.

INVESTIGATION INTO TYPE DIVISION OF MENINGOCOCCI. MARTIN KRISTENSEN and OTTO MOLTKE, *Acta path. et microbiol. Scandinav.* 2:19, 1925.

No generally applicable division of meningococci into types or groups is known. This does not mean that investigations into the mutual relationship of strains or meningococci are futile; but they ought principally to be made in direct connection with the local epidemiological questions, the strains isolated from the patients and healthy persons being compared immediately with each other, whereas the importance of investigating their relationship to so-called standard type-serums must be regarded as being problematical. The serologic investigations should be supplemented by other available methods of investigation, especially the appearance of colonies in protracted cultivation on thick ascitesagar and, perhaps, fermentation tests. Semifluid agar is an excellent medium for the maintenance of meningococcus cultures.

AUTHORS' SUMMARY.

OBSERVATIONS ON BACTERIAL INANITION. O. O. HARTOCH and C. P. MOURATOWA, *Arch. d. sc. biol.* 26:1, 1926.

Experimenting with *Bacillus paratyphosus* B and *B. proteus* X19 in conditions of inanition the authors find that morphologic changes are: first, an elongation and curving, followed by a coccoid form, and eventually a return to the original form. The coccoid form is not motile, but with a return to normal morphology motility is reestablished. In the study of agglutinogens of these organisms the thermolabile receptors are found to be lost to a large degree during the coccoid phase. Resistance is lowered and virulence practically lost in races of *B. paratyphosus* B after a period of inanition.

G. B. RHODES.

Immunology

SO-CALLED "ENDOTHELIAL BLOCKADE" WITH COLLARGOL. AN IMMUNOLOGIC AND HISTOLOGIC STUDY. FRED W. STEWART AND FREDERIC PARKER, JR., *Am. J. Path.* 2:381, 1926.

Collargol injected repeatedly intravenously in guinea-pigs depressed the formation of precipitin, depressed or stimulated the formation of agglutinin and protected against anaphylactic shock. In rabbits injected in the same way, precipitin production was depressed; there was slight or no stimulation of agglutinin and hemolysin production, but no protection against anaphylactic shock. Microscopic studies indicate that blockade of fixed endothelium is not obtained with collargol or trypan blue except in the liver and to a slight extent in the lymph nodes and marrow.

HUMAN BLOOD GROUPS: THEIR INHERITANCE AND RACIAL SIGNIFICANCE. DR. LAURENCE H. SNYDER, *Am. J. Phys. Anthropol.* 9:233, 1926.

A study of the blood groups of 200 families, involving 1,095 persons, is given, which, with the mathematical considerations of the mass data, indicates that the blood groups are inherited as a series of three multiple allelomorphs, not as two independent pairs of factors, as has long been supposed.

A detailed study of the group proportions of American Indians is given, indicating that full-blooded Indians are all of group I, the occurrence of the other groups being due to white mixture.

Evidence is presented for the normal distribution of the groups in cases of insanity, epilepsy and feeble-mindedness.

The group proportions of Maltans and Dutch are given for the first time, and additional data are presented on Americans and American negroes.

A classification of peoples, based on the frequencies of the three factors *A*, *B* and *R*, is given.

TUBERCULIN. A REPORT OF A CONFERENCE ON ITS STANDARDIZATION. *Am. Rev. Tuberc.* 14:1, 1926.

This conference was called by the United States Public Health Service, the Bureau of Animal Industry and the National Tuberculosis Association. It was prompted by a request of the Health Section of the League of Nations, addressed to the United States Public Health Service, to formulate America's standpoint in regard to an international standardization of tuberculin. The committee serving as a court of decision was composed of Dr. Theobald Smith, Dr. Ludvig Hektoen, Dr. Reid Hunt, Dr. M. Dorset, Dr. George W. McCoy and the members of the research committee of the National Tuberculosis Association. The material from which the conclusions were drawn was presented in the following way: First, the present status of the manufacture and use of veterinary tuberculin in America, Dr. E. C. Schroeder, director, Animal Experiment Station, U. S. Department of Agriculture; second, a discussion of the present methods proposed for standardization of tuberculin, Dr. E. R. Long, University of Chicago; third, the relation of serologic reactions and tuberculin activity in derivatives of tubercle bacilli, Dr. M. Pinner, Municipal Tuberculosis Sanatorium, Chicago; fourth, the principles of bacteriologic, chemical analyses and their applications to the tubercle bacillus in reference to the large scale production of tuberculin, Dr. T. B. Johnson, Yale University. The conclusions drawn were formulated as follows: We have not at present sufficient information to recommend definite changes in the standardization and testing of tuberculin as now practiced by different countries, nor shall we have until the different methods proposed have been exhaustively studied comparatively. A test based on the following definition will aid in bringing about a uniformity in practice: Tuberculin O. T. should be defined as a product derived from bouillon cultures of the tubercle bacillus (human type) by filtration and concentration and should have the following essential characteristics: (a) It should cause typical symptoms of allergy (constitutional or skin reactions) in tuberculous animals and at the same time be without effect on normal animals. (b) Its potency should be sufficient to cause the death of tuberculous guinea-pigs within twenty-four hours after intraperitoneal injection of 0.25 Gm. doses per 500 Gm. of guinea-pig weight. In practice the strength of other forms of tuberculin should be computed in terms of O. T. Tuberculin from bovine strains of tubercle bacilli should conform to the standard for O. T. Tuberculins from avian strains of tubercle bacilli do not conform to the standard for O. T. and should be considered independently. It is desirable for government bureaus concerned with licensing tuberculin to issue a statement of a method with which a product of suitable potency and purity is more or less uniformly obtainable. The commission unanimously approves the present cooperative plan of research being carried on by the Research Committee of the National Tuberculosis Association in cooperation with the Hygienic Laboratory of the United States Public Health Service and the Bureau of Animal Industry as the most likely procedure to bring about a bet-

ter understanding of tuberculin and its action, and to reach better methods of use and standardization, and the commission urges that this work be continued.

MAX PINNER.

THE DICK TEST IN SCARLET FEVER PATIENTS AND IN NORMAL PERSONS. J. SMITH and J. S. TAYLOR, *J. Hyg.* **25**:90, 1926.

The future will undoubtedly see the Dick test as firmly established in relation to scarlet fever as the Schick test is in relation to diphtheria.

AUTHORS' CONCLUSION.

THE SEROLOGIC CLASSIFICATION OF HAEMOLYTIC STREPTOCOCCI FROM CASES OF SCARLET FEVER. J. SMITH, *J. Hyg.* **25**:165, 1926.

There are probably many serologic types of hemolytic streptococci capable of causing scarlet fever. Hemolytic streptococci were obtained in throat cultures in 92 per cent of cases of scarlet fever in the first two days of the disease. The strains from 210 cases were found to belong to two main serologic types, type 1 strains in 119 cases and type 2 strains in 57 cases. Both strains belonged to Holman's pyogenes group. Only one strain (type 1) produced acid in mannite. Strains from members of the same family mainly were of the same type; this was true also of strains from cases in small, isolated outbreaks.

ON THE RÔLE OF THE RETICULO-ENDOTHELIAL SYSTEM IN TUBERCULIN HYPERSENSITIVENESS. J. FREUND, *J. Immunol.* **11**:383, 1926.

Splenectomy and injections of iron and trypan blue hinder the development of the hypersensitiveness in tuberculous guinea-pigs. Splenectomy in itself has no effect on the development of hypersensitiveness.

Injections of iron sugar and trypan blue in the amounts used do not lessen the already developed skin sensitiveness. These observations and a general consideration indicate that the reticulo-endothelial system has a function in the mechanism of the development of the tuberculin hypersensitiveness which creates an analogy between the development of the tuberculin hypersensitiveness and the production of antibodies.

S. A. LEVINSON.

IMMUNOLOGICAL STUDIES IN TUBERCULOSIS: IV. CONCERNING THE RESISTANCE TO INFECTION OF ANIMALS SENSITIZED WITH KILLED TUBERCLE BACILLI. S. A. PETROFF and F. W. STEWART, *J. Immunol.* **12**:97, 1926.

Of more than 400 guinea-pigs used in various experiments by Petroff and Stewart, there were only ninety-seven sensitized and ninety-six controls that could be utilized with certainty. Most of the animals died either at the pre-allergic or during the sensitization period, with the exception of a few that died after infection. The authors included in their series of experiments animals living fifty days or longer after infection. Fifty per cent of their animals were useless for drawing induction. Guinea-pigs were sensitized with dead tubercle bacilli, and during the allergic state, inoculations of living tubercle bacilli were made. The sensitized animals outlived the controls an average of forty-six days. Macroscopic tuberculosis was found at necropsy less extensively in the sensitized animals than in the controls. Petroff and Stewart believe that dead tubercle bacilli, only a mild skin hypersensitiveness was produced and even

that not uniformly, and no appreciable resistance could be observed in these animals. The average duration of life was approximately the same as that of the control animals.

S. A. LEVINSON.

THE NONSPECIFIC ACTIVATION IN THE COMPLEMENT FIXATION TEST OF THE ALCOHOL SOLUBLE ANTIGENS OF THE TUBERCLE BACILLUS. L. DIENES and L. D. SCHEFF, *J. Immunol.* **12**:123, 1926.

It was found that the potency of certain preparations obtained from the alcohol extract of the tubercle bacillus as antigen in the complement fixation was increased from 80 to 160 times by the addition of commercial lecithin. The potency of the original alcohol, ether and watery extract was increased considerably less (from two to ten times). Substances separated from the alcohol extract of the tubercle bacillus or cholesterin had no effect on the potency of the preparations. One millionth of a milligram of the specific substance (the dilution 1:400,000,000) in combination with lecithin as antigen was enough to cause a complete reaction in vitro.

S. A. LEVINSON.

ON THE ANTIGENIC SUBSTANCES OF THE TUBERCLE BACILLUS. L. DIENES and J. FREUND, *J. Immunol.* **12**:137, 1926.

It has been shown that beside the nonprotein-precipitable substance present in the tubercle bacillus extracts the protein substances of the extracts are potent antigens in the complement-fixation test. All three of the antigens demonstrated in the tubercle bacillus, the protein substances, the alcohol-soluble specific substance and the carbohydrate precipitable substance of Mueller and of Laidlow and Dudley, as they occur in the authors' preparations, they claim as distinct and separate antigens. A short description is given of the properties of the known antigens of the tubercle bacillus and of the response of the animal organism to them.

S. A. LEVINSON.

THE BACTERICIDAL ACTION OF THE SERUM FOLLOWING INJECTIONS OF ADRENALIN. G. H. SMITH, *J. Immunol.* **12**:205, 1926.

The intraperitoneal or subcutaneous injection of normal rabbits with epinephrine hydrochloride in quantities of from 0.5 to 2 cc. is followed by an increased capacity of the serum to kill bacteria. Large doses of epinephrine hydrochloride exert an opposite effect. The increased bactericidal action is not specific, although it is more readily demonstrated with some organisms (*Bacillus typhosus*) than with others (*Staphylococcus aureus*). The effect of the epinephrine hydrochloride becomes apparent shortly after the injection (within thirty minutes) and attains its maximum within from one and one-half to two hours. After this interval the normal potency of the serum is gradually restored, the original value being approximated within twenty-four hours. By repeated stimulation through daily injections of epinephrine hydrochloride the tendency to respond with increased bactericidal titer is diminished, suggesting that the reacting mechanism becomes exhausted or that the animal develops a tolerance to the epinephrine hydrochloride. The increase in bactericidal action is not referable to a blood concentration or to a mobilization of the known antibodies. Manifestly this effect of epinephrine hydrochloride has nothing to do with the capacity of an animal to react to artificially introduced antigen with the production of antibodies.

AUTHOR'S SUMMARY (LEVINSON).

IMMUNITY TO PNEUMOCOCCUS PRODUCED IN RATS BY FEEDING TISSUES OF ANIMALS KILLED BY THE SAME ORGANISM. V. ROSS, *J. Immunol.* **12**:219, 1926.

Rats fed on the tissues of animals killed by pneumococcus type 1 show an increased resistance to intraperitoneal injections of the same germ. The protection seems to be type specific. Protective substances exist in the serums of the immunized animals. Reference is made to the favorable immunization created by the feeding of pneumococci. An explanation is suggested for the fact that those types of pneumococci most commonly found in normal mouths are the cause of a minority of actual cases of lobar pneumonia.

AUTHOR'S SUMMARY.

IMMUNITY TO PNEUMOCOCCUS PRODUCED IN RATS BY FEEDING THEM THE GERM. V. ROSS, *J. Immunol.* **12**:237, 1926.

Rats which are fed the living pneumococci from 50 cc. of culture per day are considerably more resistant to intraperitoneal injections of the same organism than are untreated control animals. The degree of protection is as good, although not so regular, as that which was obtained when the tissues of animals killed by pneumococcus were fed. Rats which are fed the heat killed pneumococci (80 C., two hours) in the same quantities show a decidedly inferior degree of immunity.

AUTHOR'S SUMMARY.

ON THE VALUE OF THE BLOOD-GROUP FEATURE AS A MEANS OF DETERMINING RACIAL RELATIONSHIP. ELLA F. GROVE, *J. Immunol.* **12**:251, 1926.

The blood group feature of the Ainu in one district (Piratori) is very different from that of Ainu living in other districts, the former resembling the blood group feature of some Koreans, the latter resembling that of some Malay peoples.

The Sulu Moros and the Samal Moros are both Malayan people resembling each other in their outward physical characters, yet they differ widely with respect to the blood group feature. The blood group feature of the former is the same as that of the Annamese Malays, but that of the Samals is very like the blood group feature of the Hindus.

These observations, as well as similarly discordant ones that have been reported by others, reveal a serious limitation in the use of the Hirschfeld-Ottenberg method of characterizing peoples.

AUTHOR'S SUMMARY.

STUDIES IN SPECIFIC HYPERSENSITIVENESS. XXVI. ON ASTHMA AND DERMATITIS DUE TO HYPERSENSITIVENESS TO PEDICULOIDES VENTRIOSUS. ELLA F. GROVE, *J. Immunol.* **12**:263, 1926.

The "epidemic" form of bronchial asthma described by G. Ancona in Florence has been studied there with respect to the nature of the skin reactivity, and also in order to see whether the blood of the affected persons contains atopic reagins.

The cutaneous reaction was found to be different from that of atopic hypersensitiveness; (a) because it is quite inconstant and (b) because it is exhibited on surface application of the excitant.

Sensitizing bodies could not be demonstrated in the blood of either of the two sensitive persons presenting a markedly positive skin reaction.

The "epidemic" form of asthma must be placed in a special category, since it differs from the inherited form and also from the "non-sensitive" group of Cooke.

AUTHOR'S SUMMARY.

ON THE SEROLOGICAL RELATIONSHIP OF ACID-FAST BACTERIA. J. FURTH, J. Immunol. **12**:273, 1926.

There are qualitative differences in the antigenic structure of various acid-fast bacilli. These differences cannot be demonstrated clearly by the direct agglutination tests because of the complete or partial inagglutinability of most of the acid-fast bacilli.

Complement fixation and absorption experiments can be successfully applied to the antigenic analysis of this group of organisms.

Inagglutinability of acid-fast bacilli is doubtless due in part to a physico-chemical interference with flocculation, but it is probable that all receptors detectable by complement fixation are not involved in the formation of flocculi.

Mammalian tubercle bacilli have an antigenic structure different from all other acid-fast bacilli. Differentiation within the mammalian group has thus far not been found possible. One atypical bovine strain is an exception to this statement.

Avian tubercle bacilli likewise differ qualitatively from all other micro-organisms but do not form a homogeneous group for there are at least three avian subtypes. The nonchromogenic strains isolated from patients with leprosy by Kedrowsky and by Duval and the agglutinable culture of Arloing belong in this group.

A serologically distinguishable group of acid-fast bacilli from coldblooded animals does not exist. In antigenic structure these micro-organisms show little similarity to each other and to other acid-fast bacilli.

Acid-fast saprophytes differ qualitatively from all other acid-fast bacteria. Within the saprophytic acid-fast group there is at least one fixed subtype, for strains from various sources such as those designated "Smegma," "Mist," "Milk," "Pseudotuberculosis" and "Butter R" have been found to be closely related if not identical.

These observations disprove the hypothesis that acid-fast bacilli possess a common antigen present in varying proportions in various strains but show that qualitative differences occur and suggest the possibility that serologic methods may be used to identify acid-fast micro-organisms.

AUTHOR'S SUMMARY.

A STUDY OF THE LABORATORY AIDS TO THE DIAGNOSIS OF CHRONIC GONORRHEA IN WOMEN. RAYMOND S. PATTERSON, J. Immunol. **12**:293, 1926.

From the test of a limited series of cases, it may be assumed: (a) that the pus-antigen complement fixation reaction does give positive results in cases of gonorrhea in which the pus is rich in gonococci and, presumably, in antigenic substances; (b) that the reaction is specific, in that discharges from other infections did not give positive reactions; but (c) that in many chronic cases the discharges do not contain sufficient antigen to cause a reaction strong enough to be read with certainty; (d) that the pus-antigen complement-fixation

(as carried on by me) does not give as large a proportion of positive results in subacute and chronic cases as does the blood complement-fixation reaction, and hence is inferior to the later as an aid to the diagnosis of chronic gonorrhea.

AUTHOR'S SUMMARY.

HETEROLOGOUS CORPUSCULAR ANAPHYLAXIS. ROSCOE R. HYDE, *J. Immunol.* **12**: 309, 1926.

This paper furnishes proof for the theory of corpuscular anaphylaxis and disposes of the claim that the phenomenon is, in reality, due to small amounts of serum that may have been introduced with the corpuscles in the sensitizing and test doses. Sheep corpuscles sensitize guinea-pigs to chicken corpuscles, and since sheep serum does not sensitize guinea-pigs to chicken serum the condition for serum anaphylaxis are excluded.

An unsuspected difference between the rabbit and guinea-pig in their anaphylactic behavior toward these cells comes to light. Chicken cells sensitize rabbits to sheep and goat cells, but sheep cells do not sensitize rabbits to chicken cells. The converse is true of the guinea-pig. Sheep and goat cells sensitize guinea-pigs to chicken cells but chicken cells do not sensitize to sheep and goat cells.

The serums of the sheep and the goat give cross reactions in guinea-pigs, a relationship that holds for these corpuscles. The corpuscles of the sheep and goat, however, sensitize guinea-pigs for chicken corpuscles. This relationship does not hold for sheep and goat serums, for they do not sensitize guinea-pigs to chicken serum.

It is shown that anaphylaxis in the rabbit follows the behavior of the hemolytic antibody in its action toward chicken, sheep and goat cells. The phenomenon does not follow the behavior of the agglutinin toward these cells. In the guinea-pig anaphylaxis does not follow the behavior of either the agglutinins or the hemolysins toward the red cells of the chicken, sheep and goat.

The death obtained in the guinea-pigs with the red cells was typical of anaphylactic shock, as evidenced by the onset of symptoms, the violent death and autopsy observations.

AUTHOR'S SUMMARY.

A SKIN REACTION IN POLIOMYELITIS. EDWARD C. ROSENOW, *J. Infect. Dis.* **38**:529, 1926.

The absence of marked reactions in persons fully recovered from poliomyelitis and who are known to be immune; the incidence of positive reactions inversely according to age, corresponding in general to the age incidence of poliomyelitis; the strongly positive reactions during the acute stage of the disease, and the negative reaction during convalescence, are considered as presumptive evidence that the test is a measure of susceptibility to poliomyelitis.

Numerous questions regarding the nature of the reaction have not yet been worked out. However, the immune serum prepared from horses with the pleomorphic streptococcus, and used with apparent benefit in the treatment of the early stages of poliomyelitis, has a marked neutralizing power over the toxin, as determined by the skin reaction.

AUTHOR'S SUMMARY.

FURTHER STUDIES OF THE POLIOMYELITIS PRECIPITIN REACTION. EDWARD C. ROSENOW, J. Infect. Dis. 38:532, 1926.

The results of the precipitin reaction with immune horse serums and extracts of nasopharyngeal swabbings in community and institutional outbreaks of poliomyelitis, proved positive in nearly all frank and abortive cases at the time of the attack, in a high percentage of normal contacts and in persons not exposed to the disease at the time when cases occurred. It proved negative in nearly all of the cases in from two to three weeks after the acute attack had subsided and in normal persons soon after the epidemic had disappeared. In one epidemic, the incidence of positive reactions generally was found low shortly before the occurrence of the first case, high during the period of the epidemic and again low after the epidemic had subsided. The increase in positive reactions as cases of poliomyelitis developed and the decrease as poliomyelitis disappeared, occurred rapidly and seemingly independently of exposure to the disease, in isolated households in the country as well as in the urban populations. Persons who were negative to the precipitin reaction on entrance into the epidemic zone soon became positive and reactions resembling abortive attacks of poliomyelitis were common in children. The number of positive reactions in persons who came to the Mayo Clinic from widely separated communities was relatively high during the latter part of August when poliomyelitis was generally prevalent and much lower during the latter part of October after poliomyelitis had largely disappeared. After the epidemic in Rochester had subsided and the precipitin reaction in the population had become largely negative, cases occurred south of Rochester where the number of a positive precipitin reactions was high.

In certain instances poliomyelitis occurred without exposure within from five to twelve days after the presence of the streptococcus was demonstrated in the throat. Repeated swabbings showed that the carrier state lasts usually from one to three weeks in normal persons. Immunity to poliomyelitis and the occurrence of the organism in the throat did not run parallel. The positive reactions during epidemics in adults, who are relatively immune, and in children, who are relatively susceptible, were found nearly equally high, and persons who had had poliomyelitis became carriers of the streptococcus during epidemics, quite like persons who had not had the disease.

AUTHOR'S SUMMARY.

ANTIBODY RESPONSE AFTER IMMUNOTRANSFUSION IN MALIGNANT ENDOCARDITIS. KATHARINE M. HOWELL, BERNARD PORTIS and DOROTHY A. BEVERLEY, J. Infect. Dis. 39:1, 1926.

In a case of acute malignant endocarditis, both *Streptococcus hemolyticus* and *Streptococcus viridans* were isolated from the blood on three different occasions.

During a period of five months the patient received twelve transfusions of immune whole blood.

Before immunotransfusion was begun the blood serum contained no agglutinin for the streptococci isolated. The opsonic index was 0.57 and the complement-fixation reaction with streptococcus antigen was weakly positive.

The serum of the donors at the time of transfusion had an agglutinin titer of 1:10,240 or 1:20,480.

The agglutinin titer of the recipient's serum increased after each transfusion, reaching after the later ones a figure higher than that of the transfused blood.

The opsonic index of the recipient's blood serum also increased after each transfusion, as a rule running parallel with the agglutinin titer, and reaching values higher than those of the transfused blood.

Complement-fixing antibodies also increased, but appeared later than agglutinin and opsonin and fluctuated more irregularly.

The increase in specific antibodies of the recipient's blood, as compared both with its own original content and with that of the immune donors, is interpreted as evidence of the development of active as well as passive immunity as the result of immunotransfusion.

At variable periods, usually from two to three weeks, after each transfusion the agglutinin and opsonin titers of the patient's blood dropped suddenly to low figures, reaching a maximum again usually within twelve days after the next transfusion.

AUTHORS' SUMMARY.

THE BACTERICIDAL PROPERTY OF THE WHOLE BLOOD OF WOMEN INFECTED WITH THE GONOCOCCUS. GEORGE H. ROBINSON, *J. Infect. Dis.* **39**:61, 1926.

The whole blood of normal persons possesses but little bactericidal action on the gonococcus. The blood of women having a gonococcal salpingitis exerts a strong bactericidal action on the gonococcus, most marked on the infecting strain, indicating a tendency toward strain specificity. This reaction is not dependable enough with heterologous strains to warrant its use as a diagnostic procedure. Treatment of patients having chronic gonococcal infection with either stock or autogenous vaccines would seem theoretically to offer little hope of success.

AUTHOR'S SUMMARY.

TYPHOID VACCINATION BY MOUTH. VICTOR BURKE and LA VERNE BARNES, *J. Infect. Dis.* **39**:67, 1926.

Agglutinins appear in the blood of some rabbits following the introduction of standard typhoid vaccine by mouth. Artificial erosion of the intestinal mucosa in such rabbits is unnecessary.

Subcutaneous vaccination is more effective than vaccination by mouth in stimulating the production of typhoid agglutinins in rabbits.

The comparative degree of immunity to typhoid conferred by the two methods of vaccination remains to be determined. The comparative rate at which immunity develops, considered separately from the appearance of agglutinins, is also unknown. Since subcutaneous vaccination affords adequate protection against typhoid any superiority of the oral method, considered from the practical standpoint, is limited to simplicity of administration and reduction in toxicity.

The absorption of typhoid protein from the intestinal tract of rabbits apparently decreases on continued exposure of the mucosa to the protein.

AUTHORS' SUMMARY.

STANDARDIZATION OF TYPHOID VACCINE BY PHOTOMETRIC METHODS. ADELAIDE B. BAYLIS, *J. Infect. Dis.* **39**:106, 1926.

The usual method of estimating vaccines by counting in comparison with erythrocytes offers many factors of error from the outset. When the vaccine is used for purposes of immunization these errors must be taken into account.

The photometer offers precise accuracy and enables the bacteriologist to estimate vaccines with great rapidity.

The charts plotted show that there is a definite curve and by comparison of the readings for both absorption and diffusion of light an accurate estimation of the strength of the vaccine may be reached.

Photometric estimation of vaccines offers a material aid to the laboratory worker, and the photometer of Vernes, Bricq and Yvon appears to be easily adaptable for this purpose.

AUTHOR'S SUMMARY.

THE ISOLATION OF SUBSTANCES WITH IMMUNE PROPERTIES. ARTHUR LOCKE, EDNA RUTH MAIN and EDWIN F. HIRSCH, *J. Infect. Dis.* **39**:126, 1926.

The nature of the hemolysin-erythrocyte equilibrium indicates that there is in amboceptor serum a substance, probably derived from the injected antigen, which is capable of binding hemolysin and restraining its adsorption on added cells. The degree of the characteristic properties of an immune serum, such as specificity, avidity, and thermostability is shown to be dependent on the amount and character of this binding substance dispersed in the serum. The substance is adsorbed by erythrocytes together with hemolysin. When hemolysin is recovered from adsorption, its protein content is roughly equal to the sum of a constantly associated quantity of protein plus the calculated protein content contributed by the binding substance. The properties of the hemolysin may be partly changed by changing the proportion and character of its binding substance. It is postulated that antibodies are composed of clusters, of relatively large dimension, in which an elementary, naturally-occurring protein substance is adsorbed on nuclei of a binding substance derived from the injected antigen, and that they owe their individual properties to the proportion and character of this binding substance. A mechanism is suggested for the production of immune antibodies in the living organism.

AUTHORS' SUMMARY.

THE PRECIPITIN REACTION IN THE IDENTIFICATION OF SCARLATINAL HEMOLYTIC STREPTOCOCCUS INFECTIONS. EDWARD C. ROSENOW, *J. Infect. Dis.* **39**:141, 1926.

The close agreement between the specific precipitating power of the serum, prepared by the injection of suspensions and cultures of the hemolytic streptococcus from scarlet fever, and the antitoxic serum, prepared by the injection of filtrates or toxin of scarlatinal hemolytic streptococci, suggests that sufficient bacterial substance goes into solution in their cultures to make their antitoxic serum suitable for the diagnostic precipitin reaction.

AUTHOR'S SUMMARY.

PRECIPITATION WITH FRACTIONS OF SYPHILITIC SERUM AND ARACHNOID FLUID. PEARL L. KENDRICK and R. L. KAHN, *J. Infect. Dis.* **39**:202, 1926.

An attempt was made to determine which fraction of syphilitic serum and arachnoid fluid contains the reacting substances responsible for precipitation with beef heart antigen. The fractions studied were obtained by various degrees of saturation with ammonium sulphate. The precipitin reacting substances were determined quantitatively by means of the Kahn test.

The data indicate that the reacting substances are associated with the globulin fraction and not at all with the albumin, and that both the euglobulin and pseudoglobulin fractions of the globulin contain these reacting substances.

AUTHORS' SUMMARY.

DISTRIBUTION OF HUMAN HEMOGLOBIN AND BEEF LENS PROTEIN FOLLOWING A SINGLE INTRAVENOUS INJECTION IN RABBITS. LUDVIG HEKTOEN and ETHEL B. PERRY, *J. Infect. Dis.* **39**:224, 1926.

The two antigens, human hemoglobin and beef lens, as detected by anti-serums after intravenous injections in rabbits, had similar distributions. Both disappeared in about twenty-four hours depending on the amount injected; the lens substance perhaps a trifle more slowly than the hemoglobin. Hemoglobin persisted longer after injections of whole blood than after injections of pure solutions.

A considerable quantity of antigen was found unchanged in the urine, bile and intestine soon after injection. The relations between the liver and bile were less regular than between the kidney and urine, probably because of the manner of excretion and storage in the respective bladders.

The antigens were in highest concentrations in the marrow, omentum and kidneys, but seemed to be present to some extent in many tissues. The spleen became negative sooner than any other organ. After the first test one-half hour after injection there was no sign of accumulation, of selective absorption, or of prolonged retention in any organ after the disappearance from the blood.

The results with these solutions do not differ essentially from those described by others with both cellular and noncellular antigens, and the organs shown to have high concentrations of these antigens after intravenous injections are the organs that in general are known to be best equipped with cells of the reticulo-endothelial system.

AUTHORS' SUMMARY.

COMPLEMENT FIXATION IN RABIES. L. HOROWITZ-WLASSOWA, *Centralbl. f. Bakteriol.* **98**:216, 1926.

The serum of rabies infected or immunized rabbits gives fixations with the rabies virus (fixed as well as street). Fixation was not obtained with the serum of persons subjected to preventive treatment against rabies. For diagnostic purposes, the cord of animals suspected to have rabies may be tested with the serum of rabbits immunized with extracts of the salivary glands of rabid dogs. When antigen from the salivary gland is to be tested, the serum of rabbits infected or immunized with fixed virus can be used. Emulsion of fresh or glycerolated fixed virus is most effective for immunizing purposes.

POWER OF ANIMALS OF RABBIT TYPE TO FORM HETEROGENIC ANTIBODIES. I. L. KRITSCHESKY, *Centralbl. f. Bakteriol.* **99**:491, 1926.

Animals fall into two groups: (1) those of the rabbit type which can produce heterogenic antibodies but do not have any heterogenic antigens in their organs; (2) those of the guinea-pig type which possess heterogenic antigens, but cannot produce heterogenic antibodies. The rat belongs to the rabbit type.

BLOOD TRANSFUSION. P. GÖRL, *Deutsches Arch. f. klin. Med.* **151**:311, 1926.

The value of Ashby's agglutination method of determining persistence of transfused corpuscles is questioned because the number of corpuscles that were not agglutinated varied greatly as the mixture was subjected to differences in shaking.

THE POSTMORTEM SERODIAGNOSIS OF SYPHILIS. C. KRAUSPE and A. SCHMECHEL, *Klin. Wchnschr.* **5**:1611, 1926.

The Wassermann reaction and the Meinecke, Sachs-Georgi, Dold and Bruck tests were made on the blood and pericardial fluids of 1,000 bodies shortly after

death. The results were tabulated and compared with the clinical Wassermann reaction, the clinical diagnosis and the anatomic diagnosis. The conclusion is reached that the Wassermann reaction is the most reliable, the other tests corroborative only.

J. D. WILLEMS.

ETIOLOGY OF SCARLET FEVER. K. KUNDRATITZ, *Med. Klin.* **22**:1105, 1926.

Children immunized with scarlet fever toxin (Dick) were protected. Injections of toxin in sufficient doses produced all the symptoms of scarlet fever except tonsillitis.

A SERUM REACTION FOR THE DETECTION OF PRECIPITATING ANTIBODIES IN ACTIVE TUBERCULOSIS. H. LEHMANN-FACIUS and H. LOESCHKE, *München. med. Wchnschr.* **73**:1578, 1926.

A large proportion of the serums of 200 patients with active tuberculosis gave positive precipitin tests with specific organ extracts. Those of patients with arrested tuberculosis were variable. Highly labile serums of 230 tuberculosis-free patients with syphilis, carcinoma, acute infections or pregnancy, were negative.

J. D. WILLEMS.

BODY PROTEIN AND MEDICINAL ALLERGY. I. W. SAMSON and H. GÖTZ, *Ztschr. f. d. ges. exper. Med.* **52**:121, 1926.

Guinea-pigs can be sensitized with a mixture of own blood and pyramidon. In the mixture a product forms that causes sensitization.

RIECKENBERG'S REACTION IN EXPERIMENTAL RECURRENT FEVER IN MICE. WALKER KRANTZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **42**:207, 1926.

Rieckenberg observed that under the influence of specific immune serum trypanosomes become covered with blood platelets. The platelets of any mammal may be used in the reaction. The antibodies in question have been called "thrombocyto-barines" by Kritschewsky. An analogous reaction may occur when the spirochetes of recurrent fever are treated with immune serum (Brussin). Krantz confirms this observation and presents observations on the antibody concerned. Bacteria may be used in place of platelets.

ON THE HEATRESISTANT BACTERICIDAL SUBSTANCES (BETA-LYSINS) OF ANIMAL SERUM AND THE BACTERIA AFFECTED BY THEM. ALFRED PETERSSON, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **42**:233, 1926.

Heatstable bactericidal substances occur in the blood of many animals and affect many bacteria, especially anerobic. These substances consist of "activatable" and activating elements. The activating elements are of two kinds, one occurs only in certain animals and comes from the blood platelets. The "activatable" element has a pronounced affinity for the cells of the body and can be removed from the serum by short treatment with bits of fresh organs. In the case of anthrax and subtilis bacilli and probably also other bacteria, the "activatable" elements become attached to the bacterial bodies only when in the presence of the activating element. Bacteria which are affected by these heatstable substances only are not capable on injection in rabbits of inciting their production.

INVESTIGATIONS OF ANAPHYLAXIS WITH RESPECT TO LIPOIDS. ALFRED KLOPSTOCK, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **48**:97, 1926.

Guinea-pigs immunized against serum actively or passively are not affected by lipid alone. Lipoid is toxic, however, if injected shortly before or after the introduction of subfatal doses of serum. Lipoid anaphylaxis may be produced in the antianaphylactic state with respect to serum. The literature is reviewed.

SPECIFIC AGGLUTINATION OF ENTEROCOCCI (*STREPTOCOCCUS FOECALIS*). KURT MEYER and HANS LOWENSTEIN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **47**:39, 1926.

By means of the serum of rabbits immunized with nine different strains, it was found that 125 strains fell into three serologic groups. This result was confirmed by absorption experiments. Hemolytic and green-producing streptococci did not react with serum against enterococci which consequently are distinct serologically.

THE ANTIGENIC FUNCTIONS OF FORSSMAN'S LIPOID AND OTHER LIPOID SUBSTANCES. R. DOERR and C. HALLAUER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **47**:291, 1926.

Forssman's lipid, as it exists in alcoholic extracts of horse kidney, may be activated into a fully antigenic substance with specific properties by means of foreign serum, proteins of foreign erythrocytes and yeasts and bacteria. This effect is believed to be physical in nature.

THE ANAPHYLACTIC ANTIBODY AND ITS RELATIONS TO THE PROTEINS AND TO THE OTHER ANTIBODIES OF IMMUNE SERUM. R. DOERR and C. HALLAUER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **47**:363, 1926.

The globulin fraction of immune rabbit serum was found to contain the anaphylactic and other so-called antibodies, all except the amboceptor, in less concentration than the original in the serum. When the proteins of immune rabbit serum were separated into a euglobulin and a pseudoglobulin and albumin fraction by dialysis, antibodies were present in each fraction. The unitary hypothesis of the nature is upheld. The difference in antibody action does not depend on different bodies, but on the different indicators by which antigen-antibody reactions are recognized.

COMPLEMENT FIXATION BY MEANS OF COCTO-ANTIGENS FOR IDENTIFICATION AND DIFFERENTIATION OF FILTRABLE VIRUS. J. TAKAKI, A. BONIS and O. KOREF, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **47**:431, 1926.

DIAGNOSIS OF RABIC VIRUS BY COMPLEMENT FIXATION WITH COCTO-ANTIGEN AND GLYCEROL EXTRACT. R. KRAUS and J. MICHALKA, *Ibid.*, p. 504.

Boiled antigens may be used with advantage in complement fixation to distinguish between the viruses concerned in encephalitis, herpes, etc., and may prove to be of practical value in diagnostic fixation tests in cases suspected of being rabies. Emulsions are made in 0.85 per cent sodium chloride solution of brain tissue preserved in 50 per cent glycerol, and the opalescent fluid that separates on centrifugalization is used while fresh.

INVESTIGATION OF THE SIGMA REACTION OF DREYER-WARD WITH SOME CHANGES IN THE METHOD. KAJ NOREL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **47**:475, 1926.

Incubation in a dry oven appears to give better results than incubation in the water bath. The quantity of cholesterin has been increased to 0.1 cc. of a 1 per cent solution to each cubic centimeter of extract of calf's heart. In diluting the suspension more time is used and larger drops of sodium chloride solution.

QUANTITATIVE STUDIES OF ISO-AGGLUTININS WITH SPECIAL REFERENCE TO LEUKEMIA. F. SCHIFF and L. MENDLOWICZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **48**:1, 1926.

The iso-agglutinins vary in concentration; some serums are active in a dilution of from 1 to 16 only, others in dilutions as high as from 1 to 2,048. The majority have titers between from 1 to 128 and 1 to 512. The quantitative relations of the individual agglutinins in the different groups require further study. There are no differences in the agglutinin content of the sexes. The content appears to diminish after from forty to fifty years of life. In leukemia the agglutinin content is often strikingly low.

STUDIES IN CONSTITUTIONAL SEROLOGY. W. HALBER and L. HIRSZFELD, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **48**:34, 1926.

Human blood corpuscles of group A induce the formation in rabbits of lysins for sheep corpuscles and precipitins for alcoholic extracts of guinea-pig kidney more often than corpuscles of other groups. This antigenic element in corpuscles of group A is different from Forssman's antigen. The two may occur together.

ANTIBODIES AGAINST HOMOLOGOUS CIRCULATION LIPOIDS AND THEIR RELATION TO THE WASSERMANN REACTION. W. HALBER and L. HIRSZFELD, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **48**:69, 1926.

Rabbits were immunized with lipoids from rabbit red corpuscles and with swine serum. The serum of rabbits so immunized reacted with rabbit and other lipoids in complement fixation.

ISO-AGGLUTININS IN HORSE BLOOD. M. SCHWARZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **48**:79, 1926.

Iso-agglutination of horse blood is demonstrated best by mixing 0.95 cc. of citrates plasma with 0.05 cc. of a 25 per cent suspension of washed corpuscles. Horse iso-agglutinins may be active in dilutions of plasma between from 1 to 6 and from 1 to 10. Following Jansky's grouping of human blood, forty-seven of 100 specimens of horse blood seemed to fall into group 4, four into group 1 and the others partly into groups 2 and 3, leaving some specimens that could not be grouped in this scheme.

BLOOD GROUPS IN HEMOPHILIA. Wein. klin. Wchnschr. **39**:842, 1926.

Two patients with hemophilia (Kulanyi, Moritsch) both belonged to the maternal blood group. Healthy brothers belonged to different group.

IDIOSYNCRASY TO HEN'S EGG. W. JADASSOHN, *Schweiz. med. Wchnschr.* **56**:667, 1926.

The active egg substance in the case reported was dialyzable and insoluble in ether. Injection of the serum of the patient into the skin of normal persons

produced local sensitiveness manifested by hives at the place of injection when the antigenic material was injected subsequently either in the same spot or elsewhere (Prausnitz-Küstner's method).

ON THE INFLUENCE OF FAMINE REGIME ON THE FORMATION OF ANTIBODIES IN MAN AND ANIMALS AFTER VACCINATION PRACTICED IN PETROGRAD IN 1921. K. T. GLOUKHOW and J. W. SOKOLOWA, *Arch. d. sc. biol.* **26**:1, 1926.

This study of antibodies (agglutinins and complement fixation bodies) in 348 of 39,750 persons vaccinated against cholera in 1921 when food conditions were improving, as compared with similar studies on 1920, shows that the greater the degree of inanition the fewer are the demonstrable antibodies. Similar results were obtained in the vaccination of normal and starved rabbits, the antibodies also disappearing more rapidly in the latter, though the quantity of antibodies increases if a starved animal is again well nourished.

G. B. RHODES.

Tumors

DIFFUSE VERTEBRAL METASTASIS OF PROSTATIC CARCINOMA WITHOUT BONY CHANGES. WALTER M. SIMPSON, *Am. J. Roentgenol.* **15**:534, 1926.

The writer describes a case in which a clinical diagnosis of carcinoma of the prostate gland had been established. Roentgenographic studies of the lumbar spine and pelvis did not give any evidence of metastasis. At autopsy, gross evidence of spinal metastasis could not be found. Subsequent microscopic study of decalcified bone sections from the vertebral bodies revealed generalized vertebral carcinomatosis, without bone proliferation or bone destruction. The marrow substance was practically replaced by medullary carcinoma. There is a third form of osseous metastasis of prostatic carcinoma which does not conform either to the common osteoplastic, or to the less common osteoclastic, type.

AUTHOR'S SUMMARY.

ACCIDENTAL PERFORATION OF THE ATLANTO-OCCIPITAL MEMBRANE. A. T. HENRY, *Ann. Clin. Med.* **4**:682, 1926.

Henry tells of a case which emphasizes the vulnerability of the undefended atlanto-occipital space. A boy, aged 12, was accidentally stabbed, while playing with other children, with a piece of iron wire, which penetrated the atlanto-occipital membrane and almost completely destroyed the medulla at the level of the lower end of the olivary body. The case is strikingly similar to that recently described by Weller (Transfixion of the Medulla Oblongata by a Darning Needle, *Ann. Clin. Med.* **4**:107, 1925).

WALTER M. SIMPSON.

THE MECHANISM OF CANCER METASTASIS. M. T. BURROWS, *Arch. Int. Med.* **37**:453, 1926.

Metastases in cancer are not the result of a simple migration of cancer cells from the cancer to distant organs. Metastases are primarily the result of the spread of a liquid substance from the main tumor mass. This substance spreads over surfaces. It is liberated through a digestion of cells in the center of the mass of cancerous tissue. This digestion is not an autolysis resulting from the absence of oxygen, but the result of an excess of the growth stimulating substance, a product of the cell's oxidation. This fluid is rich in growth stimulating substance. This fluid stimulates not only the cancer cells to grow,

but also the normal cells. The cancer cells already adapted to it respond more quickly. In their growth they then remove the nutrition and necessary substances from the other cells and destroy them.

S. A. LEVINSON.

METABOLISM OF NORMAL AND MALIGNANT CELLS. JAMES A. HAWKINS, J. General Physiol. 9:771, 1926.

The glycolytic activity of a tissue is a function of its growth rate. Malignant tissues in most cases have a more rapid growth rate than normal tissues; in this respect they are approached only by young embryonic tissues. Tissues may be classified more closely by their anaerobic glycolytic activity than by the aerobic glycolysis respiration ratio used by Warburg.

CARCINOMA OF THE STOMACH WITH HIGH BLOOD EOSINOPHILIA. E. WEISS, J. Lab. & Clin. Med. 11:733, 1926.

The author describes a case of primary carcinoma of the stomach with high eosinophilia. Neither the tumor extract nor the blood of the patient could produce eosinophilia in animals (guinea-pigs, rabbits and dogs). Weiss could not confirm the theoretic assumptions of Koppes, who thinks that the eosinophils in the blood and in the tissues were caused by the toxins derived from necrotic tumor tissue. Not the slightest phagocytic property could be detected in the eosinophils, even in the presence of immune serum.

S. A. LEVINSON.

A CASE OF PRIMARY MELANOMA IN THE SPINAL CORD. HANS J. SCHMID, Frankfurt. Ztschr. f. Path. 33:372, 1926.

Primary melanoma of the central nervous system has been reported by many authors in the pia; a few have reported it arising from the dura or from the brain substance itself. The author reports a primary melanoma in the gray substance of the thoracic cord of a man, aged 71. There was abundant flecking of the pia of the cord with pigment. The tumor is believed to be developed from the pial chromatophores. The dura over the left convexity of the brain was also diffusely flecked with pigmented tumor. Whether this was a result of metastases from the spinal cord or a primary melanoma arising from the close relationship to the pial chromatophores is a question.

E. M. HALL.

CYSTADENOMA PAPILLIFERUM HEPATIS DEVELOPING FROM THE BILE DUCTS. ANDREAS KELEMEN, Frankfurt. Ztschr. f. Path. 33:423, 1926.

Adenoma of the liver is fairly common and usually develops from the liver cells. On the other hand, adenomas developing from the bile ducts are rare. A case of adenoma of the bile ducts is reported in a working woman, aged 69, on whom operation for a gangrenous gallbladder was performed, and who died on the third day after operation. The autopsy revealed a round tumor the size of an apple in the left lobe of the liver, enclosed in a white, rigid, fibrous capsule which was partly calcified. On sectioning a greenish yellow, partly colorless, mucous secretion ran out. Irregular masses of richly branching grayish red or grayish white, soft papillary excrescences covered the inner wall. The remaining part of the liver was normal. Microscopically the tumor was composed of a branching system of cellular connective tissue covered by epithelium of varying height. Many small bile ducts were present in the fibrous capsule and in the connective tissue framework, with many areas of round cell infiltration.

Kelemen believes that the tumor arose as the result of a chronic inflammation in which some of the bile ducts became blocked and the secretion was dammed back. Quite similar proliferation of the bile ducts is seen in the rabbit as the result of infestation with *Coccidium oviforme*.

E. M. HALL.

CEREBELLAR CYSTS. ARVID LINDAU, Acta path. et microbiol. Scandinav., suppl. 1, 1926, pp. 1-128.

More than 275 instances of cerebellar cysts have been described, and they form about 10 per cent of all cerebellar tumors. The report concerns either simple cysts or, more commonly, cysts in connection with capillary angiomas or gliomas. The angiomas are small, well defined, cortical or subcortical, mostly in the lateral or posterior parts of the cerebellum; microscopically, the picture is that of a hyperplastic, capillary angioma with pseudoxanthoma cells, small giant cells and a plasmatic transudate. Sixteen new cases are added to the twenty-four found in the literature. Four such tumors have been observed in the medulla. The gliomas vary in size; they are mostly dorsal or medial in situation; the cyst wall consists of glia tissue and, except in certain special cases, it has no ependymal lining. In angiomatosis of the retina (v. Hippel's disease) cerebellar cysts or angiomas in the cerebellum or medulla occur in about one fifth of the cases. Lindau describes one case of retinal angioma with a cerebellar cyst and angioma. In connection with angioma of the medulla and cord, cystic spaces may form that resemble syringomyelia. Lindau also discusses some fifteen cases, nine of which were observed by himself, of angiomatosis of the retina, rhombencephalon and cord; in eight of the cases a cystic pancreas was present, in ten there were cysts in the kidneys and in six there were hypernephromas. All these formations may be referred to disturbances of development in the mesoderm at about the third embryonal month. Angiomatosis of the central nervous system belongs in the same group as tuberosus sclerosis. Cysts connected with the fourth ventricle probably develop from preformed cavities. The genesis of simple cerebellar cysts is not clear; in cysts connected with an angioma or a glioma, the angioma or glioma is primary. At operation the cyst wall, as well as the tumor proper, if present, should be removed whenever possible.

Medicolegal Pathology

BLOOD TESTS FOR PATERNITY. BLEWETT LEE, Am. Bar A. J. 12:441, 1926.

The author is a lawyer in New York City. After reviewing the value of the iso-agglutinative blood test in questions of paternity, the very important question whether a person can be compelled to furnish the necessary "drop of blood" is discussed. In the case of Hayt v. Brewster Gordon & Co., Inc., it was held that "the court has power to authorize the examining physician to take a sample of the plaintiff's blood for the purpose of examination and analysis." The suit in question was one for personal injury. It is concluded by Lee that "the requirement of a drop of blood for the purpose of ascertaining paternity is within the legitimate province of the court either in civil or criminal cases, and should be made whenever the ends of justice make a blood test desirable."

THE IDENTIFICATION OF PROJECTILES IN CRIMINAL CASES. C. H. GODDARD, Mil. Surgeon 58:142, 1926.

New instruments for learning all the details about the rifling in the barrels of weapons, and for studying the marks bullets received from the barrels are

described and illustrated. Emphasis is given to the importance of impressions on the head of the shell made by the breech block at the time of firing, to the marks due to ejector apparatus and to the topographic interrelations of such marks on the shell. Comparative examinations of shells and bullets are made by using two microscopes with a common eyepiece; photomicrographic methods are also used.

The report comes from the Bureau of Forensic Ballistics, a branch of the Bureau of Criminal Science, independent organizations assisting the police of New York city in suppression of crime, and serving without remuneration.

The bureau is making a large collection of small arms and ammunition of all varieties from all countries—a real museum of weapons, including old revolvers and pistols no longer manufactured. It is of interest that many of the cheaper weapons of this kind in common use in this country are made in Spain.

The article does not contain any reference to other similar scientific studies, such, for example, as that by Raestrup (*Deutsche Ztschr. f. d. ges. gerichtl. Med.* 7:242, 1926).

E. R. LeCOUNT.

PATHOLOGIC ANATOMY OF ACUTE AND CHRONIC MORPHINE POISONING.

W. WEIMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 8:205, 1926.

Dark fluid blood in the heart and lungs, engorged vessels in the abdominal organs, brain and meninges, and, more rarely, hemorrhages in the brain and myocardium are the gross changes found after death from acute morphine poisoning. Like the changes due to most poisonous alkaloids, they are in no sense specific. They are usually encountered when death results from suffocation, no matter how produced.

When morphine or opium poisoning is chronic, there is thickening of the bronchial and intestinal mucous membranes from long-standing passive hyperemia, also edema of the lungs, some anasarca, hypodermic abscesses and the pigmented scars and amyloid disease caused by the abscesses. These alterations are also encountered when death results from other causes.

Weimann obtained material for study from the bodies of two persons dying from chronic poisoning, one from morphine and one from laudanum and also from two other bodies with death following acute morphine poisoning, at the end of ten hours in one instance and at the end of two and one-half days in the other. He also used material from guinea-pigs, rabbits and mice experimentally poisoned. His studies were chiefly microscopic, especially of the brain. Detailed accounts are said by him to be forthcoming in the *Zeitschrift für die gesamte Neurologie und Psychiatrie*.

In animals and man, there is a pronounced fatty change in the cerebral ganglion cells. In the human brain this change is in the ganglion cells of Ammon's horn and the deeper layers of the cortex of the frontal lobes. In animals as the phylum is descended, and the development and differentiation of the cerebral cortex is less pronounced, the fatty changes are increasingly in the ganglion cells of the corpus striatum and deeper parts, and the ganglion cells of the cortex are unaffected. In both man and the animals examined, ganglion cells of the medulla and spinal cord, and the nerve fibers, generally were not altered.

In the human cerebral cortex, with this marked fatty change, there is in chronic morphine poisoning an atrophy which stimulates the pitting following infarction. It is caused by fatty degeneration in the walls of small arterioles and by the changes in them which occur as sequences to this change. Accom-

panying this focal atrophy of the frontal lobes, the leptomeninges at such places are adherent and thickened.

Fatty degeneration is also pronounced in the renal epithelium, heart muscle and liver, and to a less degree in the spermatogenic cells of the testis. There are reports of lipuria from fatty changes in the renal epithelium in dogs experimentally poisoned. The accumulation of fat in all these places occurs in both acute and chronic morphine poisoning. The fat may disappear leaving the cells honeycombed as in hydropsical degeneration. This Weimann noted especially in the liver. A similar change has been found in conditions accompanied by greatly lowered nourishment and in metabolic diseases. Weimann believes the fatty degeneration he found is not accompanied by mobilization of fat from other places, such as is said to occur in poisoning from phosphorus, poisonous mushrooms and chloroform.

It has been assumed for a long time that the human frontal convolutions and the structures composing them represent a recent evolution, and as a consequence are prone to suffer injury like other highly specialized structures. The changes described by Weimann in the brain are also encountered as a result of the poisonous action of alcohol. He makes no reference to this relation between highly specialized structure and susceptibility to disease, but his account of alterations due to morphine in the liver when taken in conjunction with our general knowledge of its retrogressive changes and their causes, suggests that the human liver, as at present constituted, may be as highly specialized and differentiated as the frontal cerebral convolutions.

E. R. LeCOUNT.

ON NEW MEDICOLEGAL PROBLEMS. H. ZANGGER, Schweiz. med. Wchnschr. 56:687, 1926.

The author deals with two phases of medicolegal work: namely, (1) accidents and deaths in garages, due to volatile poisons; (2) the criminal, civil and compensation demands for alcohol determinations.

Many are ignorant of the demands of the law on medicine to determine volatile, decomposable substances in cadavers. Problems and peculiar situations often arise, because of this volatility, and remain unsolved. Deaths due to volatile poisons at present far outnumber those of the solid and liquid poisons.

The fact is often overlooked that substances like carbon monoxide and alcohol are excreted, are chemically changed in the body during life and are decomposed after death; that changes in the blood pigment due to nitric oxide and to carbon monoxide may be mistaken for each other; that putrefactive products may simulate the alcohol reactions; that fruit juices, essences, camphor, aldehydes and phenols may interfere with the alcohol reactions; that foreign substances from gloves, vessels and air may be absorbed by the material under investigation. All of these may interfere with the alcohol determination and then have an important bearing on the legal aspect. Negative chemical observations do not necessarily mean that death was not due to carbon monoxide. The carbon monoxide may have been lost through the respiration during the last interval of life, by putrefactive processes after death, or because the method employed for its determination was not sensitive enough.

The author then emphasizes the importance of bringing the medical facts and history of the case in all detail in correlation with the chemical observations. All of these points must be studied carefully in each case before a conclusion is arrived at which may conscientiously be submitted in court.

A case of carbon monoxide poisoning, illustrating these points, is cited.

On the question of garage accidents due to volatile poisons, Zangger deals solely with tetra-ethyl lead. This extremely poisonous substance, originally made to be used as a war gas, is now advocated as an antiknock substance to be used in gasoline. He calls attention to the danger to chauffeurs, garage workers and the public in general. He believes an industry should be prohibited to use such a dangerous substance, especially as there is not a general need for the product and since it could be replaced by some nondangerous substances.

A. O. GETTLER.

Technical

CEREBROSPINAL FLUID IN THE NEWBORN. A. LEVINSON, J. GREENGARD and R. LIFVENDAHL, *Am. J. Dis. Child.* **32**:208, 1926.

Cerebrospinal fluid was obtained from 100 cases of suspected cerebral hemorrhage in newborn babies, by spinal or cistern puncture. Cistern puncture was found to be easier and productive of more fluid with a lower incidence of traumatic blood contamination. In the depression just below the external occipital protuberance, a 20 or 22 gage, 1½ inch (3.8 cm.) needle with stylet was introduced anterior, then directed caudad to pass the lower occipital crest, and then anterior until just through the atlanto-occipital ligament and dura. The pressure was found to average 20.5 mm. of mercury higher by this method than by spinal puncture. The observations were not diagnostic of hemorrhage.

W. P. BLOUNT.

THE SUCCESSFUL APPLICATION OF THE CULTURE METHOD TO THE DIAGNOSIS OF INTESTINAL FLAGELLATES IN THE FIELD. CLAIRE McDOWELL HILL, *Am. J. Hyg.* **6**:646, 1926.

It is well known that trophozoites of *Chilomastix mesnili* and *Trichomonas hominis* become quiescent and disappear from the stools a few hours after they are passed. Cases of chilomastixiasis may often be diagnosed by the presence of cysts in the smear. For the diagnosis of an infection caused by *Trichomonas*, however, one is dependent on the recognition of the trophozoites. For this reason, in surveys and routine examinations in laboratories, often cases of trichomoniasis are not found or the positive cases are so few as to be considered of no importance. In the experiments reported here, 912 stools were examined by the smear and culture methods. By the smear method the rate of infection with *Trichomonas* was 1.7 per cent. By the culture method the rate was raised to 9.2 per cent. On these same specimens the rate of infection with *Chilomastix* was 5.7 per cent by the smear method and 9.4 per cent by the smear and culture methods combined.

This study, therefore, has led to the conclusion that examinations by the culture method are practical under field conditions and are more efficient. For *Trichomonas*, which has no known cyst stage, the method is particularly of value. For *Chilomastix* the culture method in combination with the smear method gives much better results than the smear method alone.

The medium used was the Hogue modification of the ovomucoid medium. The whites of three eggs were shaken thoroughly in a flask with glass beads, and 600 cc. of a 0.7 per cent solution of sodium chloride was added. The mixture was cooked for fifteen minutes over a boiling water bath and was shaken constantly while cooking. It was allowed to cool slightly and was filtered first through a coarse wire strainer and then through cheese cloth.

As soon as it was filtered, it was put in tubes in 5 cc. samples. As an autoclave was not available, the tubes of medium were sterilized by boiling in a pan of water for three successive days. If the tubes were to be used within the next three or four days, they were boiled only once for a period of about thirty minutes.

Although the medium was prepared in the same way each time, the final products varied. As a rule, after the tubes of medium had stood for a short time, a white precipitate separated, leaving a shallow cloudy layer of supernatant fluid. Occasionally, however, the precipitate did not settle, and the medium remained opaque and like a jelly throughout. It was found, as pointed out by Hegner and Becker, that *Chilomastix* grew with difficulty on a medium of this type. Therefore, such a batch of medium was always discarded. In the course of my experiments from twenty-five to thirty lots of medium were prepared, and not more than three or four were successful.

The fecal material was transferred to the tubes by means of a toothpick. The end of the toothpick was inserted into various parts of the fecal sample until an amount about the size of a small pea had been collected. The toothpick and fecal material were then dropped into the test tube medium, and the tube was kept in the incubator for twenty-four hours. The incubator was an old kitchen oven the temperature of which was raised by an electric bulb kept burning constantly inside. The temperature of the oven varied between 29 and 36 C., though usually it did not go below 32 C., even during the night.

STUDIES ON THE QUANTITATIVE ESTIMATION OF THE TOTAL PROTEIN CONTENT IN CEREBROSPINAL FLUID. G. A. YOUNG and A. E. BENNETT, *Am. J. M. Sc.* **172**:249, 1926.

* The total protein content of cerebrospinal fluid is estimated by reading the volume of the sediment produced after precipitation with alcohol, acetic acid readings vary from 25 to 75 Gm. per hundred cubic centimeters. The highest readings are obtained in the meningitides, central neuritis, xanthochromic fluids and general paralysis. Definite evidence of organic neurologic disorder may be detected by the new method in fluids which give only a normal or doubtfully positive qualitative globulin test.

ARTHUR LOCKE.

A NEW TABLE FOR LACTOSE (MILK OR URINE) AND GLUCOSE (BLOOD OR URINE) CALCULATION WITH NOTES ON THEIR ESTIMATION. H. D. HASKINS, *Am. J. M. Sc.* **172**:256, 1926.

A complete table is furnished which gives a direct reading of the percentage of sugar (glucose of blood and urine, also lactose of milk and urine) corresponding to the cubic centimeter of the special sodium thiosulphate solution used for titration. This table can be used only with the author's modification of the Shaffer-Hartmann method. Lactose of milk or urine can be estimated easily and accurately with the same reagents and technic as are used for the estimation of glucose in blood and urine.

AUTHOR'S SUMMARY.

DYE METHODS FOR DETERMINING THE BLOOD VOLUME TESTED IN VITRO. J. LINDHARD, *Am. J. Physiol.* **76**:497, 1926.

The object was to determine to what extent, if any, injected dyes were retained by the plasma colloids, and to what extent they became adherent to the red cells. His experiments as recorded were limited to vital red. Aside

from errors inseparable from hemolysis when present, it was found that the blood of different species behaved differently. With undiluted ox-blood there was a constant systematic error, proportional to the red cell volume. The error diminished with dilution beyond the proportional decrease in red cell volume. Adsorption of the dye by the red cells was demonstrated. In undiluted dog's blood there was likewise an error associated with the presence of red cells, but not increasing proportionally to their volume. In samples of (female) human blood, a systematic error was not detected, although the samples did not contain more than 36 per cent of the red cells in any case. Inconvenience did not result from repeated injections of dye.

H. E. EGGERS.

SPECTROPHOTOMETRIC DETERMINATIONS OF BILIRUBIN. C. SHEARD, E. J. BALDES, F. C. MANN and J. L. BOLLMAN, *Am. J. Physiol.* **76**:577, 1926.

The writers have studied, and discuss, the effects of fading and of slight degrees of turbidity and hemolysis on spectrophotometric determinations of bilirubin in plasma and serum. In the absence of fading the laws of Lambert and Beer apply accurately to the absorption of light by bile, with wave lengths of 430 to 500 microns. By the use of the spectrophotometric method bilirubin may be determined to one-fiftieth of the smallest amount measurable by the Van den Bergh method.

H. E. EGGERS.

A SIMPLIFIED METHOD FOR THE CULTIVATION OF *ENDAMOEBIA HISTOLYTICA*. CHARLES F. CRAIG, *Am. J. Trop. Med.* **6**:333, 1926.

Locke solution containing one part to seven of human, horse or rabbit serum, heated to 56 C. for thirty minutes, is a good medium for *Endamæba histolytica*. The Locke solution contains:

Sodium chloride	9.00 Gm.
Calcium chloride	0.24 Gm.
Potassium chloride	0.42 Gm.
Sodium bicarbonate	0.20 Gm.
Dextrose	2.50 Gm.
Distilled water	1,000 cc.

After filtration the solution is autoclaved at 15 pounds (7 Kg.) pressure for fifteen minutes; the serum is now added and the mixture filtered (Mandler or Berkefeld) until clear. The reaction need not be adjusted as the ameba always grows within its p_H limits. *Endamæba histolytica* has remained alive and motile in this medium for eleven days; subcultures were obtained after eight days; successive subcultures at intervals of twenty-four and forty-eight hours have been secured over three months, without change in form or power to reproduce.

HEMOGLOBIN, COLOR INDEX, SATURATION INDEX AND VOLUME INDEX STANDARDS. E. E. OSGOOD, *Arch. Int. Med.* **37**:685, 1926.

The term "hemoglobin coefficient" is proposed as a substitute for "the amount of hemoglobin in grams per hundred cubic centimeters of blood calculated to a red cell count of 5 million per cubic millimeter." This is the figure used as 100 per cent hemoglobin in calculating color indexes. Similarly, the term "volume coefficient" is proposed to take the place of "the volume of packed red cells in cubic centimeters in 100 cc. of blood calculated to a red cell count

of 5 million per cubic millimeter." This is the figure used as 100 per cent cell volume in calculating volume indexes. The term "saturation index" was proposed by Haden to express the ratio between the average hemoglobin content per unit volume of cells in the blood examined and the average hemoglobin content per unit volume of cells in the blood of normal persons of the same sex in the same age group. The author's method of calculating saturation indexes by dividing the color index by the volume index is simpler than Haden's method of calculation. The hematocrit is not a reliable substitute for hemoglobin estimations or for red cell counts. The use of oxalated venous blood (20 mg. of powdered potassium oxalate per 10 cc.) is advised for all work, including red cell counts. A tentative classification of anemias on the basis of color index, volume index and saturation index is suggested.

S. A. LEVINSON.

A CRITICAL EVALUATION OF HAHN'S QUANTITATIVE METHOD FOR DETERMINING PROTEIN AND PROTEOSE. F. B. SEIBERT, *J. Biol. Chem.* **70**:265, 1926.

The Hahn method for the determination of protein and proteose consists in precipitating aliquots of the serum with (1) 2.5 per cent trichloroacetic acid, and (2) phosphotungstic acid. The nitrogen of the second precipitate is non-protein, nonproteose nitrogen. That of the first precipitate is, supposedly, non-protein nitrogen. The method, with some modifications, is 99 per cent accurate.

ARTHUR LOCKE.

A COLORIMETRIC METHOD FOR THE DETERMINATION OF ACETONE BODIES IN BLOOD AND URINE. J. A. BEHRE and S. R. BENEDICT, *J. Biol. Chem.* **70**:487, 1926.

A colorimetric method is described for the determination of the acetone bodies in normal or pathologic urine or blood, based on the reaction of acetone with salicylic aldehyde in alkaline solution.

AUTHORS' SUMMARY.

FORMED ELEMENTS IN URINARY SEDIMENT OF NORMAL INDIVIDUALS. T. ADDIS, *J. Clin. Investigation* **2**:409, 1926. EFFECT OF SOME PHYSIOLOGIC VARIABLES ON NUMBER OF CASTS, RED AND WHITE BLOOD CELLS AND EPITHELIAL CELLS IN URINE OF NORMAL INDIVIDUALS. *Ibid.*, p. 417.

Addis gives the averages and ranges of variation in the twelve hour rates of excretion of casts, red and white blood cells and epithelial cells in urine from seventy-four normal persons. The averages of eighty-two counts on these subjects were: casts, 1,040; red blood cells, 65,750; white blood cells and epithelial cells, 322,500. The type of cast found was the hyaline variety. There were only three instances in which a single epithelial or granular cast was discovered. The hyaline casts only rarely contained any cell inclusions, and fat droplets were never seen.

Quantitative determinations of the number of formed elements in the urine of normal persons failed to show that either bodily movements of various types or the ingestion of a large amount of protein in the form of meat had any statistically significant effect.

THE USE OF ISOPROPYL ALCOHOL IN THE PREPARATION OF WASSERMANN ANTIGENS. R. M. ISHAM, *J. Lab. & Clin. Med.* **12**:52, 1926.

Isopropyl alcohol is regarded as superior to ethyl alcohol for the preparation of Wassermann antigens of the simple alcoholic type. It yields a product of superior antigenic power and shows relatively less anticomplementary action.

Isopropyl alcohol is superior also for the production of acetone insoluble antigens because it gives a more complete extraction of the acetone insoluble lipoids of normal tissues. The antigen produced is equal in antigenic power to that produced by use of ethyl alcohol and is somewhat superior as regards anticomplementary behavior with normal serum.

S. A. LEVINSON.

QUANTITATIVE TEST OF BILE SALTS IN BLOOD. P. SZILÁRD, *Biochem. Ztschr.* **173**:440, 1926.

The blood plasma is extracted with alcohol and the bile acid salts precipitated with ether. The precipitate, when dissolved in distilled water, will react with ferric chloride proportionally to the amount of bile acid salts in solution. This product dissolved in alcohol gives a bright red reaction with sulphosalicylic acid, and is compared with the standard made up in the same way from the sodium salt of glycocholic acid. The method is very sensitive, and gives a positive reaction in the blood of healthy persons. It can also be used to test duodenal contents.

COUNTING BLOOD PLATELETS. F. B. HOFMANN, *Deutsche med. Wchnschr.* **52**:861, 1926.

Tyrode solution, with the addition of one fifth of its volume of a 0.1 per cent solution of mercuric chloride, proved to be the best fluid for preserving platelets. In healthy persons the counts average 760,000 in men and 682,000 in women.

THE CLINICAL SIGNIFICANCE OF THE GAS EXCHANGE DETERMINATION. A. LUBLIN, *Klin. Wchnschr.* **5**:1263, 1926.

The gas exchange determination (Rahel Plaut) is not a sure means of differential diagnosis between obesity of thyrogenic and hypophysial origin.

ARTHUR LOCKE.

BREEDING OF HOOKWORMS. K. USAMI, *Wien. klin. Wchnschr.* **39**:595, 1926.

The stool is mixed with charcoal and incubated in a Petri dish; after three or four hours agar is poured on the surface and a little water added; the larvae pass through the agar and are found in the water.

CALCIUM CHLORIDE AS A PRESERVATIVE FOR SHEEP CORPUSCLES. W. E. HILGERS and T. WOHLFEIL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **47**:337, 1926.

For the preservation of sheep corpuscles a 0.1 per cent solution of calcium chloride in an 0.85 per cent solution of sodium chloride is recommended. It is believed that this solution is better than any other preservative now used.

THE DIAGNOSIS OF TUBERCULOSIS, BY GOLDENBERG'S MODIFICATION OF BESREDKA'S TECHNIC. H. SCHULTE-TIGGES, *Ztschr. f. Tuberk.* **45**:212, 1926.

This technic of complement fixation uses the hemolysins and the complement in the patient's blood. A certain parallelism exists between the hemolytic activity of the serum and the extent of the lesion; serums which are strongly positive have usually a higher hemolytic titer. The diagnostic efficiency of the test appears to be about the same as that of Besredka's original test.

MAX PINNER.

Society Transactions

THE SOCIETY OF AMERICAN BACTERIOLOGISTS

28th Annual Meeting, Philadelphia, Dec. 28, 29, 30, 1926

Hans Zinsser, President; J. M. Sherman, Secretary

SECTION ON GENERAL BACTERIOLOGY

HANS ZINSSER, Chairman

STUDY OF THE GROWTH OF *CLOSTRIDIUM BOTULINUM* AND *CL. SPOROGENES* IN VEAL INFUSION BROTH UNDER REDUCED PRESSURES. GAIL M. DACK, W. A. STARIN and MARIE WERNER, Department of Hygiene and Bacteriology, University of Chicago.

Cl. botulinum types A and B and *Cl. sporogenes* were inoculated into tubes of veal infusion broth, which were placed under reduced pressures of air, oxygen and carbon dioxide. The tubes were sealed under the reduced pressures and incubated at 37 C. The growth of the organisms and the toxin production of *Cl. botulinum* were studied under these conditions. Both spores and vegetative cells were used.

At air pressures of 4 cm. and less growth was regular, and in most cases occurred within one to two days. At pressures greater than 4 cm. growth was irregular, and in some cases occurred after several months. Growth was not obtained in any tube at pressures greater than 16 cm.

The growth of spores and vegetative cells did not show marked difference under reduced air pressures. Toxin was not demonstrated in greater dilutions when 0.8 cm. of pressure was employed than when 5 cm. was used.

Hydrogen peroxide was not demonstrated by Avery's method in any of the tubes tested.

At oxygen pressures of 5 and 10 cm. growth was not noted after three months' observation.

Growth of the organisms occurred regularly and uniformly at carbon dioxide pressures as high as 50 cm. Pressures higher than this were not employed. Growth was not any greater in tubes which were first evacuated and then placed under atmospheric pressure of carbon dioxide before finally reducing the pressures than in tubes which were merely evacuated and the final carbon dioxide pressure maintained.

Cultures tested after one, five and thirty day periods of growth showed toxin production to be at its maximum after five days. The highest count of organisms was obtained after the one day period of growth.

The hydrogen ion concentration of the cultures remained within the limits of growth for each organism tested throughout the entire experiment.

THE ISOLATION OF PATHOGENIC ANAEROBES FROM AFRICAN POISONED ARROWS. IVAN C. HALL, University of Colorado.

The literature on poisoned arrows indicates that they owe their fatal action not only to various specific poisons but also in part to pathogenic micro-organisms. It is well known that arrow wounds which are not fatal within a

few minutes or hours are likely to become the seat of serious infections, notably tetanus, gaseous gangrene and cellulitis. Yet bacteriologic studies of such wounds have been few, and I do not find any studies of the bacterial flora of the arrows themselves in the available literature.

This study deals with six arrows made by African bushmen and brought back to America by the Denver African Expedition of 1925, which was headed by Dr. C. E. Cadle. Three of these arrows belonged to the Kalahari bushmen, two to the Heikum tribe and one to the Ovachimbos of the Kaokoveldt. Only the Heikum arrows yielded poison; this was a dark, dry, gummy substance, and, according to Dr. Richard Whitehead, belongs to the group of glucosides, of which ouabain is an example, found in certain species of *Strophanthus*. A small guinea-pig was killed in six minutes by 10 mg of the crude poison.

All of the arrows except the smaller Heikum arrow had pathogenic bacteria on their points. Besides several species of "hay bacilli," there were isolated and identified the nonpathogenic *B. centro-sporogenes*, *B. bifermentans*, *B. sporogenes*, two species of sporulating anaerobes belonging to obscure or possibly new species, *Staphylococcus albus*, *Streptococcus fecalis* and *Streptococcus mitis*. The pathogens were *B. histolyticus*, *B. novyi*, *B. septicus*, and *B. welchii*; all of these were typically virulent for guinea-pigs. Special tests for *B. tetani*, *B. botulinus* and *B. coli* failed to reveal any of these germs.

QUANTITATIVE ASPECTS OF THE PROTEIN METABOLISM OF *C. PUTREFACIENS* (McBRYDE). L. B. PARSONS and W. S. STURGES, Laboratory of the Cudahy Packing Company, Omaha, Nebr.

A complete study has been made of the organism first isolated by McBryde (U. S. Dept. Agric., Bur. Animal Ind. Bull. 132, 1911). The formol and ammonia nitrogen produced by *C. putrefaciens* in meat and gelatin mediums has been determined as a function of time. These values show this organism to be markedly proteolytic, although other common putrefactive anaerobes are shown to digest more rapidly and to produce greater amounts of ammonia and amino-acids. Volatile acids produced during proteolysis consist of from 85 to 90 per cent acetic and from 10 to 15 per cent isovaleric. Nonvolatile acids are not produced. An exact equivalence between volatile acid and ammonia has been demonstrated for six strains at all ages and for twenty-four strains at one age. Preliminary data indicate that this equivalence does not exist in the case of several other anaerobes. These data admit of certain conclusions of a fundamental nature concerning the mechanism of deamidization.

A STUDY OF SOME ANAEROBIC MICRO-ORGANISMS FOUND IN NASOPHARYNGEAL WASHINGS. SARA E. BRANHAM, Department of Hygiene and Bacteriology, University of Chicago.

Nasopharyngeal washings from twenty-six persons were examined by the technic of Olitsky and Gates. These twenty-six washings represented eight normal persons, thirteen with colds and five with cases of acute uncomplicated influenza within thirty-six hours after the onset. From eleven of these samples seventeen strains of anaerobic micro-organisms were isolated, fourteen of which seemed to belong to species previously described. Five strains seemed to be identical with *Staphylococcus parvulus* reported first by Veillon and Zuber, and since found abundantly by Holman and Krock and by Hall and Howitt. Two were probably the spirochetes described by Tunncliffe. Seven were apparently the filtrable micro-organisms reported by Olitsky and Gates: Two of these

seemed identical with their group I, one with group III. Four were probably either *Bacterium pneumosintes* or group II; three of these came from cases of influenza.

The three remaining strains seem to be hitherto undescribed. One of these, a small hemolytic diplococcus, which grew only under anaerobic conditions, is described here.

ON THE CULTURAL CHARACTERISTICS OF *LACTOBACILLUS ACIDOPHILUS*. C. ROOS, H. K. MULFORD COMPANY, BIOLOGICAL LABORATORIES, GLENOLDEN, PA.

The name *L. acidophilus* applies to a group of biologically related strains variable in cultural and morphologic characteristics.

The changes noted in a strain under close observation for four years strongly indicate that the various types of *L. acidophilus* colonies, as found on plating feces, are mutants.

Acid production, carbohydrate fermentation and colony formation are not constant characteristics, and alone do not constitute a basis for classification.

From the standpoint of clinical usage, source of the strain, nutrient and physical requirements to facilitate growth and temperature range are important factors, and must necessarily be considered.

POTATO UTILIZATION AS INDICATED BY HYDROGEN PEROXIDE. JEAN GÜLL and JEAN BROADHURST, Teachers College, Columbia University.

The dissatisfaction commonly felt with solid potato as a diagnostic medium is due not only to the irregularity with which growth occurs on potato for a given species, but to the variations in appearance due to the differences in the softening of the potato in sterilizing, the height of the water in the tube and the "browning" and drying of the potato slices. Therefore, we have adapted a hydrogen peroxide test being used in our laboratory to this situation, adding 1 cc. of hydrogen peroxide to twenty-four-hour (loop) cultures of the organism in (5 cc.) semifluid potato, stirring the mixture thoroughly after releasing the hydrogen peroxide at the bottom of the tube. The results with some thirty species of bacteria may be classified in several qualitative groups: (1) no ebullition of gas, as in sterile mediums, (2) a mere ring of surface bubbles, (3) a frothy layer of about one-fifth to one-fourth the height of the potato-hydrogen-peroxide mixture, (4) a foamy mass about equaling the height of the mixture, and (5) a foamy mass from two to almost four times the height of the mixture, as in *Serratia marcescens* and *Pseudomonas pyocyaneus*.

These qualitative groups are merely tentative; and this report is but a preliminary one, made in the hope of securing cooperation (especially cultures) for other phases of hydrogen-peroxide reaction (namely, effect on pigment manifestations, bacterial relationships) now being carried on in our laboratory.

APPARATUS SUGGESTIONS: (1) TEST TUBE PLUGS, (2) SEMIPOROUS PETRI DISHES AND (3) CENTIMETER COUNTERS. JEAN BROADHURST, Teachers College, Columbia University.

(1) *Test Tube Plugs*.—To decrease the labor and expense attendant on the usual rolled cotton plug, a hollow rubber plug has been devised. The hollow is loosely filled with a small mass of absorbent cotton, insuring sterility, but readily meeting the usual air and steam adjustments; each end of the plug curves slightly toward its central opening, causing the slightly bulging sides to press tightly against the test tube. A surfacing of paraffin, if desired, is

easily applied, and it interferes less with the future manipulations of the plug than do cotton plugs. If preferred, a covering of absorbent cotton can be used on these rubber plugs; its application requires but a single manipulation, in which the cotton center is also placed in the plug cavity.

(2) *Semiporous Petri Dishes.*—In an effort to secure a cheap semiporous Petri dish cover, several different chemical substances, such as cellulose acetate, and commercially known "fillers" and lacquers have been tried on the unglazed white covers.

(3) *Centimeter Counters.*—The delimitation of given areas on slides for direct counting of bacteria (vaccines, milk) and on Petri dishes for colony counts may be more satisfactorily made by applying pieces of gummed paper, with cut-out openings of 1 and 2 sq. cm. Such cut-out pasters are preferable to outlines with a wax pencil, because they are exact and fragments of pencil do not confuse the microscopic count. In counting crowded Petri dishes, several advantages are found: the cut-out paster fits close to the glass surface, and changes in the angle of the worker's head causes less variation in the size of the field actually counted; the Petri dish can be held up in front of the eye, while the worker may assume a comfortable position which can be varied at will, instead of bending in a cramped position over the Stewart or Wolffhuegel plate. The margin of the paster is wide enough to be used for recording results, and it acts as an eye shield, especially in the lantern-counting of colonies previously described by the author. The 2 sq. cm. counters are recommended for direct milk counts for which a thinner film is desired than is obtained with the 1 sq. cm. area. Counters for trial use will be supplied on request.

A PRELIMINARY STUDY OF TWO HUNDRED GRAM-NEGATIVE BACILLI ISOLATED FROM CASES OF GENITOURINARY INFECTION. J. H. HILL, L. R. SEIDMAN, A. M. S. STADNICHENKO and M. G. ELLIS, James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore.

This paper is a study of the cultural characteristics of 200 cultures of gram-negative bacilli isolated from genito-urinary infections. The methods used included the fermentation of carbohydrates, growth in gelatin, the production of acetyl-methyl-carbinol, the methyl red test, indol production, motility, urea decomposition, hemolysis, capsule formation and citrate utilization in Koser's citrate broths and on Simmons citrate agar.

From these observations the organisms were divided into five main groups as follows: group 1, organisms not producing acetyl-methyl carbinol, methyl red positive, fermenting lactose and other carbohydrates with the production of acid and gas, citrate utilization none or scant; group 2, organisms producing acetyl-methyl carbinol, methyl red negative, fermenting lactose and other carbohydrates with the production of acid and gas, citrate utilization prompt; group 3, *Proteus*; Group 4, *Pseudomonas pyocyanea* and group 5, organisms not in groups 1, 2, 3 or 4.

Under group 2 a study of the rate of production of acetyl-methyl-carbinol has been made, under group 3 the decomposition of urea has been studied especially, the fermentative action of group 4 has been emphasized.

These organisms, especially groups 1 and 2, have been compared with each other and with organisms previously reported by others in genito-urinary infections. Comparisons have also been made with similar organisms isolated by others from different sources, such as the intestinal tract, soil and milk. Emphasis has been placed on the pathogenicity of the organisms in group 2

of our series. A correlation of the organisms studied with the clinical observations is included.

SURFACE TENSION STUDIES WITH *L. ACIDOPHILUS* AND *L. BULGARICUS*. NICHOLAS KOPELOFF and PHILIP BEERMAN, Department of Bacteriology, Psychiatric Institute, Ward's Island, N. Y.

A method of standardization has been developed which yields fairly concordant results in growing these lactobacilli at varying surface tensions. Using sodium ricinoleate and sodium oleate as depressants, nine different strains of *L. acidophilus* (six of these isolated from commercial *Acidophilus* products now on the market) and six different strains of *L. bulgaricus* have been tested repeatedly in different concentrations.

All strains of so-called *L. acidophilus* with one exception were able to grow at a surface tension of 3 or more dynes lower than any *L. bulgaricus* in the presence of sodium ricinoleate. The average critical point for *L. acidophilus* strains for this depressant was 37.9 dynes, while the average critical point for *L. bulgaricus* strains was 5.2 dynes higher, or 43.1 dynes.

It is significant that two strains of *L. acidophilus* which have been passed through the human intestinal tract, and are of proved therapeutic benefit, had the lowest critical point, namely, 35 dynes.

Both *L. acidophilus* and *L. bulgaricus* strains grew at lower surface tension when sodium oleate was used as a depressant than when equivalent concentrations of sodium ricinoleate were employed.

These results substantiate the contention that surface tension is a satisfactory criterion for differentiating *L. acidophilus* from *L. bulgaricus*.

THE EFFECT OF SODIUM RICINOLEATE AS A SURFACE TENSION DEPRESSANT ON THE GROWTH OF BACTERIUM COLI. W. R. ALBUS, Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

A study has been made of the effect of sodium ricinoleate as a surface tension depressant and as a salt, on the growth of a strain of *Bacterium coli*. The results of these experiments are presented.

THE EFFECT OF BACTERIAL CELLS ON THE CHEMICAL COMPOSITION OF THE SURROUNDING MENSTRUUM AS INFLUENCED BY THE PRESENCE OF VARIOUS ELECTROLYTES. H. J. SHAUGHNESSY and C-E. A. WINSLOW, Yale University School of Medicine.

Cells of *Bact. coli* in the zone of physiologic interest (p_H 4-10) produce changes in acidity by means of direct absorption of H or OH ions in such a manner that there may not be any injury to the cell. Sodium chloride and calcium chloride decrease the ability of the cells to perform this action, most markedly in the alkaline range.

In addition to this type of regulation, there is another which seems to be dependent on the production of carbon dioxide and ammonia, aided perhaps by diffusion of hydrogen ions carried into the surrounding fluid by chloride or phosphate ions.

More prolonged exposure of bacterial cells to aqueous menstrooms leads to an overproduction of ammonia, and in the case of *B. cereus*, which dies under the conditions of the experiment, a marked excess of alkali is produced.

Living cells of *Bact. coli* and even dead cells of *B. cereus* appear to be relatively impermeable to chlorine phosphate and calcium ions. On the other hand,

carbon dioxide and ammonia pass freely. The cell wall of *B. cereus* is in general much more permeable than that of *Bact. coli*.

Neither heating the cells at 60 C. for fifteen minutes nor boiling for thirty minutes affects either the diffusion of electrolytes or the liberation of carbon dioxide. Boiling, however, almost wholly inhibits the production of ammonia.

Dilute solutions of sodium chloride promote the liberation of the substances studied from both normal and heated cells.

Strong solutions of sodium and of calcium, on the other hand, decrease the liberation of ammonia and other alkaline substances, but increase, relatively, the liberation of acidic substances.

In the case of *Bact. coli*, strong solutions of calcium show a sharp initial rise in titratable alkalinity and in titratable acidity followed by a fall, a phenomenon we attribute to a decreased permeability which leads to lysis and liberation of proteins followed by an accumulation of new reactive films on the protein micellae or to absorption of the oppositely charged ions.

THE INTERPRETATION OF CHANGES IN ELECTRICAL RESISTANCE ACCOMPANYING THE DEATH OF CELLS. A. ZOOND, MacDonald College, Quebec, Canada.

The electrical resistance of suspensions of *Bacillus cereus* in balanced Ringer solution is found to decrease when the suspension is killed by heat or by mercuric chloride. The resistance measurements, however, indicate that the phenomena accompanying the death of the cell by heat and by mercuric chloride are not identical.

When cells killed by mercuric chloride are resuspended by repeated centrifuging in Ringer solution, it is found that the resistance of the suspension is the same before and after death. This clearly indicates that the conductivity of bacterial cells, namely, their resistance to the passage of ions moving under the influence of an electric current, is not materially affected by the death of the cell. The pronounced drop in resistance accompanying the death of the cells under the influence of mercuric chloride can be accounted for only by the assumption that death is accompanied by destruction of the semipermeable membrane surrounding the bacterial cell, and that consequently there is an adjustment of osmotic differences which may modify the concentration of salts in the suspending solution, and thus affect its electrical resistance.

When a suspension of *Bacillus cereus* in Ringer solution is killed by heat, there is a pronounced drop in the resistance of the suspension. This, however, is accompanied by a corresponding drop in the resistance of the suspending solution, indicating that the resistance of the cells is not materially affected by the heat treatment, but that the decrease in resistance is due to the diffusion of salts out of the cell. This view is supported by the fact that when the suspending solution is replaced by a solution of normal concentration by means of repeated centrifuging the resistance of the suspension again rises to a value of little below the resistance of the living suspension. That it does not quite attain that value is explained by the observed fact that the volume of the cells is materially decreased by the heat treatment. This is further supported by the observation that when a suspension killed by mercuric chloride is subjected to heat, there results a further drop in resistance, which remains constant through repeated centrifuging and resuspensions in Ringer solution.

The data presented in this paper show that, in interpreting the results of resistance measurements, the essential difference between the permeability of cells to ions moving under the influence of an osmotic gradient and their permeability to ions moving under the influence of a potential gradient must

be fully recognized. Measurements of resistance give valuable information as to the osmotic changes occurring in the cell solution system, but the permeability of the cell ions moving under the influence of an electric current does not seem to be affected in any way by the phenomenon of death.

A MODIFICATION OF THE BROWN APPARATUS FOR THE DETERMINATION OF COLORIMETRIC p_H . WILLIAM H. WRIGHT and H. G. HARDING, College of Agriculture, University of Wisconsin.

An arrangement of the p_H scale on the glass background according to the range of this indicator makes a more convenient set, and permits comparison and cross-checking of indicators. Clark and Lubs buffer solutions sterilized intermittently at 100 C. have been found to be entirely satisfactory and to retain their accuracy.

LIMITING FACTORS IN THE LACTIC FERMENTATION. L. A. ROGERS and E. O. WHITTIER, Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

Cultures of *Strept. lactis* increase to a level which is constant for the same culture under fixed conditions. At this level there does not appear to be appreciable increase or death of cells, but fermentation continues for some hours.

This level has a direct relation to the buffer content of the medium, is higher in milk than in broth, higher in milk or broth maintained at a constant p_H , and still higher in mediums held at a constant p_H and aerated. Holding the p_H constant prolongs the fermentation, but it finally ceases. Reducing the crowding effect by filtering and returning filtrate does not prolong multiplication or fermentation. The effect of concentration of undissociated lactic acid in stopping acid production was found to vary somewhat with other factors, as indicated by earlier investigators. In an aerated milk culture the reduction potential is raised from approximately -0.2 volts to $+0.2$ volts. The oxidation-reduction level probably affects the population level.

BACTERIAL METABOLISM. THE INFLUENCE OF PHOSPHATE BUFFER IN SUGAR-FREE AND IN GLUCOSE-CONTAINING MEDIUMS. C. A. SLANETZ and LEO F. RETTGER, Laboratory of General Bacteriology, Yale University.

Metabolism experiments were conducted on the following twenty-two organisms: *B. subtilis* (two strains), *B. cereus*, *B. megatherium*, *B. anthracis*, *Bact. coli* (two strains), *Bact. typhosum* (two strains), *Bact. paratyphosum* A and B (two strains each), *Ps. pyocyanea*, *E. prodigiosus*, *Bact. bronchisepticus*, *Bact. pullorum* (two strains), *Proteus vulgaris*, *Staph. aureus*, *C. sporogenes* and *C. welchii*.

The studies were concerned with changes effected in amino-acid nitrogen, nonprotein nitrogen, ammonia nitrogen, biuret, H-ion concentration, glucose and turbidity in buffered and in unbuffered plain and glucose broth.

The presence of balanced phosphate (1 per cent or M/7) in carbohydrate-free and in glucose-containing peptone had an accelerating effect on bacterial metabolism. This influence is directly associated with the maintenance of suitable H-ion concentration.

Marked differences in nitrogen metabolism were observed between different types or groups of organisms, and even between species of the same genus, as for example, *B. cereus* and *B. megatherium*.

The "proteolytic" activity of some organisms may be inhibited or greatly retarded by the presence of fermentable sugar in the medium. However, this inhibition is not due directly to the sugar itself, but to the accumulation of products of carbohydrate metabolism, the organic acids.

When favorable environmental conditions are maintained, as, for example, by phosphate buffer, the type and degree of nitrogen metabolism exhibited by an organism in glucose broth may approximate those displayed by it in plain peptone broth.

Definite, though usually small, amounts of amino-acids and ammonia are utilized by bacteria during the period of active growth. A greater decrease in amino and ammonia nitrogen generally accompanies the more luxuriant growths noted in buffered glucose-containing mediums than in the unbuffered and in the carbohydrate-free mediums during the period of rapid multiplication.

When *B. cereus* is grown under conditions of greatly reduced oxygen supply, ammonia formation is slight, but the changes in amino-acid nitrogen compare favorably with those brought about under fully aerobic conditions.

STUDIES ON THE METABOLISM OF THE BACT. ABORTUS-MELITENSIS BRONCHISEPTICUM-ALCALIGINES GROUP. NITROGEN METABOLISM. JAMES G. MCALPINE and CHARLES A. SLANETZ, Storrs Agricultural Experiment Station, Conn.

Eight strains of *Bact. abortus*, five of bovine and three of porcine origin, five strains of *Bact. melitensis* from various sources, two strains of *Bact. bronchisepticum* and two strains of *Bact. alcaligines* were employed in this study. The organisms were grown in 1 per cent Fairchild's peptone broth, both with and without the addition of 1 per cent glucose. Duplicate sets were grown in the presence of 10 per cent carbon dioxide and in jars containing ordinary air. After incubation at 37 C. for two, six, nine and fourteen days, the broth cultures were analyzed for free ammonia, for amino nitrogen by the Van Slyke and Sørensen methods, for nonprotein nitrogen and for the utilization of glucose by the Benedict method. The rate of growth was measured by the McFarland nephelometer, and hydrogen ion concentrations were determined by the colorimetric method of Clark.

The results of these preliminary experiments are briefly given. Carbon dioxide accelerated the growth of the bovine strains of *Bact. abortus*, even though they had become accustomed to aerophilic conditions by long continued culture on artificial mediums. Added carbon dioxide did not stimulate growth of the porcine strains. The growth of *Bact. melitensis*, *Bact. alcaligines* and *Bact. bronchisepticum* was markedly retarded by the addition of 10 per cent carbon dioxide.

Hydrogen ion determinations showed constantly increasing p_H values for *Bact. alcaligines*, *Bact. bronchisepticum* and bovine strains of *Bact. abortus*, even in the glucose-containing medium. In glucose broth, *Bact. melitensis* and the porcine strains of *Bact. abortus* constantly exhibited p_H values approximating, or lower than, those of the controls. This may be accounted for by the apparent utilization of *Bact. melitensis* and the porcine strains of *Bact. abortus* of small quantities of glucose as determined by the Benedict method. This apparent utilization was not so marked in the bovine strains of *Bact. abortus*, in the cultures of *Bact. alcaligines* or in those of *Bact. bronchisepticum*. It has been assumed that certain nitrogen fractions tend to give high values in the Benedict test, and it is possible that the utilization of these substances by the bacteria in question would result in lower sugar values.

Bact. alcaligines, *Bact. bronchisepticum* and *Bact. abortus* of bovine origin showed a steady increase in free ammonia in the glucose broth during the fourteen

days' incubation. On the other hand, the porcine strains of *Bact. abortus* exhibited little if any change in free ammonia content. In the cultures of *Bact. melitensis* relatively little free ammonia could be demonstrated before the ninth day.

There was comparatively little change in the amino-nitrogen content of the cultures of *Bact. melitensis* and the swine strains of *Bact. abortus*. The bovine strains of *Bact. abortus* utilized considerable amounts of amino-nitrogen during the fourteen-day period of observation. This utilization also was marked in the cultures of *Bact. alcaligenes* and *Bact. bronchisepticum*.

From these preliminary experiments there appears to be a decided difference in the metabolic activities of *Bact. melitensis* and *Bact. abortus* of bovine origin, at least in the strains used in this study. Cultures of *Bact. abortus* from porcine sources resembled more closely *Bact. melitensis* than the bovine strains of *Bact. abortus*.

A BIOCHEMICAL METHOD OF DIFFERENTIATING BRUCELLA ABORTUS FROM BRUCELLA MELITENSIS — PARAMELITENSIS. FOREST HUDDLESON and ELIZABETH ABELL, Bacteriological Section, Michigan Agricultural Experiment Station.

It has been found that *Brucella abortus* in its growth activity on a suitable medium, under aerobic incubation, causes the liberation of hydrogen sulphide gas while *Brucella melitensis* or *Brucella paramelitensis* does not. The gas may be detected by means of lead acetate paper.

In this study ninety-two strains of *Br. abortus*, thirty strains of *Br. melitensis* and ten strains of *Br. paramelitensis* were employed. All of the strains were isolated either in Europe or in this country from bovine, swine, human, caprine and equine sources.

The hydrogen sulphide test so far has agreed with the agglutination absorption test in placing the strains in the abortus or melitensis group. The hydrogen sulphide test offers a rapid procedure for the grouping of newly isolated strains.

STUDIES IN BACTERIAL METABOLISM: ON THE SELECTIVE UTILIZATION OF AMINO-ACIDS BY BACTERIA. E. LEE TREECE, Department of Bacteriology, University of Kansas, Lawrence, Kans.

The utilization of a number of amino-acids by members of the intestinal group of bacteria was studied by means of growth rates and by a comparison with the utilization of cystine in a synthetic medium. Hydrogen sulphite production was used as an index of the utilization of the cystine by the organism.

In this way it is shown that when two amino-acids are present, one may be used in preference to the other. Such sparing of cystine serves to explain the delayed hydrogen sulphide production from peptone by certain bacteria of this group.

A BLOOD-CLOT DIGEST MEDIUM FOR CULTIVATION OF HEMOPHILIC AND OTHER BACTERIA. R. S. SPRAY, School of Medicine, West Virginia University.

It is believed that the medium herein described preserves the valuable features of chocolate agar, while it eliminates the tedious details of the aseptic preparation required for routing chocolate agar.

After removing serum from blood clot for preparation of Loeffler's medium the clot is drained and comminuted by passing through wire gauze. It is then boiled, and again finely divided. One liter of the semifluid material is placed in a 2 liter flask; 20 Gm. of anhydrous sodium carbonate, 5 Gm. of trypsin and 15 or 20 cc. chloroform are added.

The fluid is incubated for fifteen days, with a second addition of 3 Gm. of trypsin on the tenth day. Chloroform may be added on the fifth and tenth days to prevent bacterial decomposition.

By the fifteenth day digestion is practically complete. The fluid is rendered strongly acid with hydrochloric acid (50 to 75 cc.) and steamed in water-bath to drive off chloroform, then titrated with normal sodium hydroxide to reaction p_H 7.4 to 7.5; it is then placed in tubes and autoclaved. If the tubes are sealed with paraffin, the medium keeps indefinitely.

This blood clot digest, thus prepared similarly to Wolf's casein-digest, may be added in from 5 to 10 per cent to melted North's, or other agar, placed in tubes, and sterilized like plain agar. On the resulting medium, *H. influenzae*, *Pneumococcus*, and *Streptococcus* grow abundantly. When sodium oleate is added, as in the Avery medium, *H. influenzae* grow vigorously.

If the blood clot digest is filtered through cotton, a clear filtrate is obtained, which, added to agar, gives a clear medium similar to hemolyzed blood agar, on which *H. influenzae* colonies are characteristic.

BACTERIOPHAGE ACTIVE AGAINST A THERMOPHILIC BACILLUS. STEWART A. KOSER, Hygienic Laboratory, University of Michigan, and the Department of Bacteriology, University of Illinois.

A lytic principle, or bacteriophage of d'Herelle, active against a thermophilic organism was obtained from river water polluted by sewage. The organism in question is a slender spore-forming rod. Its optimum growth temperature appeared to be from about 45 to 52 C., and development at this point was more rapid than at 37 C. The maximum temperature was from 58 to 60 C. At 20 to 25 C. the culture developed slowly, requiring several days to produce scanty growth on agar slants. A lytic filtrate was developed by the usual method of alternate feeding and filtration at 37 C. After development at this temperature it was found to be active against the homologous culture at various points from room temperature (20 to 25 C.) up to about 58 C., with pronounced and vigorous action at 50 to 52 C.

The filtrate fulfilled the commonly accepted tests for demonstration of the bacteriophage. It caused both inhibition and lysis of the culture, was transmissible in series, and in appropriate dilutions formed plaques of lysis on agar slants previously inoculated with the culture. At 50 to 52 C., the plaques were larger than at 37 C. Tests for inactivation showed that this "thermophilic bacteriophage" survived heating at 70 C. for thirty minutes, but was destroyed at 75 C. Thus, in spite of its activity at higher temperatures, its heat resistance is about the same as that reported for the usual coli, typhoid or dysentery bacteriophage.

AN ADAPTATION OF THE CHAMBERS MICRODISSECTION APPARATUS FOR THE ISOLATION OF SINGLE BACTERIAL CELLS. WILLIAM H. WRIGHT and ELIZABETH F. MCCOY, College of Agriculture, University of Wisconsin.

The double Chambers apparatus as made by Leitz has been found to be well adapted to the isolation of single bacterial cells. The 13 mm. chamber made by Leitz is easily converted into a moist chamber in which drops of sterile solutions as small as from 5 to 10 microns can be held without evaporation for over an hour. Single cells are isolated in such a small drop with one pipet, and transferred to sterile culture medium with the other.

Two types of micropipets have been found useful. One is a 45 degree type for making the small drops, and one a 90 degree type for removing single cells.

Neither should have a tip bore of less than 5 or more than 10 microns for bacteria, and 25 to 30 microns for yeasts.

Advantages of the technic are: certainty of single cell cultures; ease of manipulation—six isolations in thirty minutes under best conditions, while the average is about three per hour; the isolation of pure cultures when selective cultural methods often fail; the fact that single cell isolations have given growth with about 90 to 100 per cent of young yeast cells, 25 to 30 per cent with aerobic bacteria and 1 per cent with anaerobic bacteria.

SYMPOSIUM ON FILTRABLE VIRUSES

HANS ZINSSER, *Chairman*

FILTRABLE VIRUSES—A CRITICAL REVIEW. T. M. RIVERS, Rockefeller Institute for Medical Research, New York City.

Throughout this discussion the term "filtrable viruses" is used in a noncommittal way to designate active transmissible agents which are capable of producing pathologic conditions in bacteria, plants, insects, fish, birds, and mammals, and which by general consent are limited for the moment to the number of active agents connected with the diseases listed in the table. The arrangement of the diseases is for convenience of discussion, and does not carry a classificatory significance. In the first place, filtrability of the etiologic agents does not sharply delimit this group of diseases, as it is well known that the viruses share this characteristic with certain small bacteria and vibrios, and also with some spirochetes and protozoa. Furthermore, in regard to the etiologic agents of some diseases within the group, either filtration experiments have not been recorded, or there is much discussion as to whether they are filtrable. In the past all attempts to classify these diseases have been unsuccessful, and there is every reason to believe that such attempts are still premature. The diseases listed form a heterogeneous group. In fact, they exhibit so many differences that a discussion of the filtrable viruses almost amounts to a separate discussion of each disease. This is due to the fact that the filtrable virus group has been used to a considerable extent as a dump heap for infectious diseases of unknown etiology. Therefore, it is not unlikely that some of them will be shown to be caused by small bacteria or protozoa. When this occurs, such diseases should be removed from the filtrable virus group, and given their correct position in the classification of diseases.

Epidemiology.—Epidemiologic problems presented by the virus diseases in regard to regional distribution, seasonal variation, host susceptibility and virulence are similar to the problems found in connection with other infections.

Immunity.—It is doubtful, with a few possible exceptions, whether injection of a virus completely inactivated leads to a protection against the same virus in an active state. Diseases produced by the filtrable viruses usually lead to a lasting immunity if the patient recovers. In this respect virus diseases differ from those caused by ordinary bacteria. This is not universally true, however, since one attack of typhoid fever produces a fairly lasting immunity. Enduring immunity has never been explained satisfactorily. Whatever the true cause of the phenomenon may be, it is not unlikely that it is closely related in some way to the peculiar parasitism exhibited by the viruses.

Prevention of Virus Diseases.—The prevention of virus diseases depends on the protection of susceptible persons from exposure or on their immunization by means of vaccination with attenuated or modified viruses.

Filtrability.—Since the discovery of the first filtrable virus in 1892, it has been determined by means of different kinds of filters that many diseases are caused by active agents smaller than ordinary bacteria. Some of them are presumably much smaller, and are probably optically immeasurable. Others, however, do not seem to be so small, and concerning their filtrability there is much discussion. Methods of filtration are crude and inaccurate. The most one can say concerning the viruses is that under given experimental conditions they

Filtrable Virus Diseases

Bacteriophage

Mosaic diseases of plants
(infectious chlorosis)

Sacbrood

Wilt of European nun moth

Wilt of gypsy moth caterpillar

Jaundice of silk worms

Epizootic of guinea pigs

Hog cholera

Cattle plague (Rinderpest)

Pernicious anemia of horses

Virus III infection of rabbits

Foot-and-mouth disease

1 Type A

2 Type O

Vesicular stomatitis of horses

Paravaccinia No report on filtration

Trachoma and inclusion blenorrhoea

Infectious papular stomatitis of cattle

Molluscum contagiosum

Warts

Contagious epithelioma (fowl-pox)

1 Chickens

2 Pigeons

Infectious myxomatosis of rabbits

Rous sarcoma of chickens

Leukemia of chickens

Lymphocystic disease of fish

Epithelioma of fish

Carp-pox

No reports on filtration

Mumps (According to Karmount, a spirochetal disease)

Agalactia (According to Bridg, a bacterial disease)

Salivary gland disease of guinea pigs

Measles (rubeola)

German measles (rubella) (No report on filtration)

Grippe (influenza) (According to Orlitzky and Gates a bacterial disease)

Common colds

1 Swinepox

2 Goat pox

3 Sheep pox

4 Horse pox

5 Cowpox

6 Smallpox

7 Varioloid

8 Alastrim

9 Chicken pox

10 Herpes zoster

11 Encephalitis

12 Lethargic

13 Following vaccination

14 Japanese 1924

15 Koritschoner

16 Australian X disease

17 Poliomyelitis

18 Rabies

19 Born's disease

20 Fowl plague and plague of blackbirds

21 Guinea pig paralysis

22 Distemper of dogs

A Insect-borne

B Nairobi disease of sheep

C Catarrhal fever (blue tongue) of sheep

D African horse sickness

E Pappataci fever

F Dengue fever

G Yellow fever (According to Noguchi a spirochetal disease)

H Typhus fever

I French fever

J Rocky Mountain spotted fever

K Heartwater disease

L Flood fever of Japan

M Bacterial diseases

N Oroya fever and verruga peruviana

O Pleuropneumonia

P Avian diphtheria

Q Scarlet fever

Majority of the diseases which have been placed in the filtrable virus group by different workers.

either pass or do not pass through certain filters. The failure to pass a filter, however, is not determined in every instance by the size of the virus. Filtrates from uncontrolled, and even from well controlled, sources may contain more than one active agent, some of which may be cultivated on artificial mediums. All investigators should be extremely careful in working with filtrates not to be misled by their observations, and ascribe to an active agent an etiologic rôle in a disease with which it has nothing more than an accidental connection.

Size.—The size and weight of molecules of crystalline egg albumin and crystalline hemoglobin are not agreed on. Consequently, what hope is there at present of ascertaining the size of viruses which have not been obtained in a pure state?

Cultivation.—It has not been proved that any of the etiologic agents of the diseases in the table down to mumps can be cultivated in the absence of living cells. A satisfactory explanation of the difficulty experienced in cultivating the viruses on artificial mediums is not easily found. Their small size alone should not necessarily make them insusceptible to cultivation. Nor does it seem to be a question of delicacy or sensitiveness, because many of them are extremely resistant to chemical and physical agents. Furthermore, viruses are not found multiplying free in nature. The viruses appear to be obligate parasites in the sense that their reproduction is dependent on living cells.

Cell Types in Relation to Virus Reproduction.—In view of the fact that most viruses multiply only in the presence of living cells, it is advisable to ascertain what kinds of living cells promote their reproduction best, and what effect on the cells is induced by the reproduction. A remarkable specificity of species is exhibited by many viruses. Frequently young cells seem essential for the activity of the viruses. Some viruses show an affinity for cells of certain tissues, and apparently can neither multiply nor produce signs of disease unless they come into close relation with these cells. It is not known whether the viruses multiply intracellularly or extracellularly. Nevertheless, they have a profound influence on cells, and cause remarkable changes within them. This influence probably accounts for the fact that in lesions caused by many viruses intracellular changes assume appearances characteristic enough to be spoken of as inclusion bodies. In this respect many diseases caused by viruses differ from those caused by ordinary bacteria.

Inclusion Bodies.—Inclusions may occur in the cytoplasm, in the nucleus or in the cytoplasm and nucleus. Various ideas are held concerning them. Some investigators consider them merely as products of degeneration, but others believe that they are the virus itself, while yet others think of them as virus surrounded by a mantle of altered cellular material. As yet their nature has not been definitely determined. Nevertheless, in spite of the ignorance concerning their nature, inclusion bodies have held and will continue to hold an important position in the study of this group of diseases. Many attempts to produce significant inclusions by artificial means have been unsuccessful. Therefore, under properly controlled conditions, the presence of inclusions, accepted as significant, is undoubtedly in the majority of instances indicative of the presence of a virus in the immediate vicinity.

Characteristics of Filtrable Viruses.—A wide range in the degree of resistance to physical and chemical agents is exhibited by the viruses. The question of the organized or corpuscular nature of viruses has not been satisfactorily settled. Bacteriophage, rabies virus and herpes virus, in the absence of living cells, do not respire enough to be detected. Phenomena have been observed that cause one to ask whether viruses can mutate. Whether it is correct to speak of these phenomena as examples of mutation is not known. In any event, when viruses are adapted to alien hosts, their characteristics are frequently altered, as well as those of the diseases produced by them. It is impossible to say at present whether the viruses are animate or inanimate. In regard to the filtrable viruses, it can be said that they exhibit, when compared one with another, a diversity of characteristics equal to, if not greater than, that exhibited by ordinary bacteria and other known forms of life. Consequently, one should not generalize when dealing with this remarkably heterogeneous group of active agents.

Identity of the Epitheliotropic and Neurotropic Viruses.—In the table, the diseases from swinepox through rabies have been arranged and bracketed in a way that quickly shows the relation claimed by different workers to exist between members of the group. Many of the viruses may be closely related, or some may have evolved from a common ancestor, yet it is unlikely that all claims in regard to the identity of these viruses are correct. More experiments are needed to settle some of the debated questions.

Summary.—In the majority of the diseases caused by viruses there exists a close relation between the etiologic agents and the cells of the hosts. This peculiar type of parasitism may account for the fact that some of the diseases show a striking specificity in species, that the viruses have evaded cultivation on simple mediums, that characteristic or specific pathologic changes are frequently observed in cells affected by viruses, and, finally, that a host once recovered from a virus disease usually possesses a lasting immunity.

THE NATURE AND SIGNIFICANCE OF CELLULAR INCLUSION BODIES IN DISEASES.
DUE TO FILTRABLE VIRUSES. E. V. COWDRY, Rockefeller Institute for Medical Research, New York City.

From the cytologic point of view the inclusion bodies which are developed in rabies, herpes, vaccinia, chickenpox, and several other diseases listed in this rather ill defined group are of great interest. They represent reactions on the part of the cells to influences of which little is known. These reactions are often highly specific and absolutely without counterpart, to the best of our knowledge, in any other physiologic or pathologic conditions.

The inclusions themselves, which occur not only in man and in many vertebrates, but also in certain insects and plants, are characterized by great diversity. For this reason generalizations are difficult to make, and are often stultified by the number of qualifications and exceptions which must be noted. Yet, to attempt to envisage the series of reactions, as Findlay and Ludford (1926) have done, seems worth while. Nuclear inclusions, as one would expect, are less frequently associated with disease than cytoplasmic ones, and are also less varied in character, because the nucleus occupies a protected position, being, in large measure, sheltered from the environment by its investment of cytoplasm, and because its chemical constitution is more restricted. It cannot be doubted that the incidence of inclusions, both nuclear and cytoplasmic, is in reality a question of the permeability of a peculiar group of substances, some of them remarkably stable in character. The inclusions, with but few exceptions, exhibit the property of growth by accretion. In some cases they are formed largely, or in part, by a kind of overproduction of material, traces of which may be observed antecedent to the action of the viruses. Obviously *Rickettsiae*, which have sometimes been included in this category, belong elsewhere. Information regarding these specific inclusions is in a sense superficial. Single types of inclusion have not been studied with the concentration that has been lavished on a zymogen granule of the pancreas, for example. Many methods of cytologic study devised within the past decade have apparently never been applied. The attention of specialists has but rarely been invoked. Biochemists interested in cellular physiology have much to offer. Great refinements in cytologic technic have been perfected in the study of the nucleus, and yet investigators who have devoted their lives to a better understanding of the nucleus have never felt impelled to study these truly remarkable nuclear inclusions. The inclusions in plants have been studied by botanists, those in insects by entomologists and those in higher forms by physicians and pathologists. Recognition that the problem is common to all makes for progress.

RECENT RESEARCH ON FOOT AND MOUTH DISEASE, WITH SPECIAL REFERENCE TO THE WORK OF THE AMERICAN COMMISSION. HARRY W. SCHOENING, Bureau of Animal Industry, Washington, D. C.

A brief review of the recent research work on foot and mouth disease with special reference to the work of the Commission appointed by the U. S. Department of Agriculture to study foot and mouth disease is given. Active work was done by the Commission at Strasbourg and Alfort, France, in addition to an epidemiologic study of the disease in various countries.

The disease was transferred to guinea-pigs and carried through 261 passages. The virus after numerous guinea-pig passages was still highly active for larger animals. Attempts at cultivation of the virus were unsuccessful. The work of Frosch and Dahmen could not be confined. A study of factors enhancing survival of the virus in artificial mediums indicated that hydrogen ion concentration of $p_H = 7.5$ or 7.6 , and temperature less than 37°C .; the optimum being room temperature, were essential, while anaerobic atmospheric conditions were found to be more favorable than aerobic. The best medium was of the simplest kind and of a semisolid nature, 0.25 per cent agar or 10 per cent gelatin. At room temperature the virus in such mediums remained alive for more than sixty-nine days but less than one hundred. The virus appeared not to multiply in vitro.

Under field conditions, in hay or garden soil, the virus was found active for at least twenty-five to thirty days.

By means of cataphoresis the electric charge of the virus was found to be positive, the iso-electric point being $p_H =$ about 8. The virus was regularly filtrable through Seitz asbestos disks, Berkefeld V candles and Chamberland bogies L-1 to L-5. Some adsorption of the virus occurred in Berkefeld N and the Chamberland L-7 and L-9 bougies, while in the L-11 bougie it was completely retained. The virus being electropositive and the filters electronegative, adsorption occurred in the denser filters, owing to oppositely charged materials. When the charge of the virus was changed to negative, it readily passed through the Chamberland L-11 bougie. Positive filtrates were also obtained with the most porous of Bechold's ultrafilters.

The virus was found active in dilutions as high as 1:10,000,000. The size of the virus has been delimited by molecular filtrations to be between 20 microns and 100 microns in diameter, indicating that it is particulate and not of a fluid character.

The abnormal resistance of the virus to such antiseptics as alcohol, acetone, mercuric chloride and compound solution of cresol is explained by the precipitation of protein materials by these chemicals, as a result of which the virus is protected by the coagulum. When this precipitation is prevented, 60 per cent alcohol, for example, will kill the virus in less than one minute. Sodium hydrate or antiformin, which do not cause precipitation, readily destroy the virus.

It was found by experiments on guinea-pigs, cattle and hogs that at least two types of foot and mouth disease virus exist, thus confirming the work of Vallée and Carré. The disease produced by these viruses is indistinguishable clinically, but they do not cross-immunize.

Under rigid experimental conditions the presence of a carrier could not be demonstrated in twenty cattle recovered from foot and mouth disease and especially selected by Swiss officials as being probable carriers of the virus. Field evidence on this point is strong, however, although the percentage of animals acting as carriers is believed to be small.

The hoofs of twenty-two cattle and one hog were examined at postmortem twenty days to six months after the onset of the disease. Scrapings were made

of sawed sections, and guinea-pigs were inoculated. One positive result was obtained in a cow thirty-four days after infection.

The earthworm could not be implicated as a carrier.

Cattle were found to contain active virus in the saliva before any lesions of the disease were observable. Tests on guinea-pigs showed materials taken from infected cattle to be infectious not longer than seven days after the first appearance of symptoms. In most cases, however, such materials were inactive after four days.

Immunity follows an attack of foot and mouth disease, and is of two types: local, or tissue, and general, or blood. The tissue immunity is the first to disappear, and as a rule may be said to last at least from four to six months, and the general immunity from one to two years, although much shorter and longer periods have been recorded. Limited attempts at artificial immunization were unsuccessful.

By means of guinea-pig tests antibodies can be demonstrated in immune and hyperimmune serums.

Hyperimmune serum from cattle showed about the same potency as so-called reconvalescent serum. By repeated injections of virus into rabbits a more potent serum was obtained. Normal horse serum, or serum from a horse repeatedly injected with virus, did not show protective properties. Horses were resistant to infection with both types of foot and mouth disease virus. Complement-fixation tests were uniformly negative.

A comparative study was made between vesicular stomatitis and foot and mouth disease. A marked similarity was found in the two diseases, the distinguishing features being a lack of cross-immunity, the susceptibility of the horse to vesicular stomatitis and its resistance to foot and mouth disease.

SOME CHARACTERISTICS OF VIRUS DISEASES OF PLANTS. L. O. KUNKEL, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.

Virus diseases of plants are systemic maladies, in most cases producing marked chlorosis. They are caused by filter-passing agents. They produce two general types of disease. In the first type, chlorosis is noticeable in all chlorophyll-bearing tissues, and diseased plants are yellowed. In the second type, chlorosis is confined to certain areas irregularly distributed in the green tissues so as to produce a color pattern characteristic of the so-called mosaic diseases. Both types cause stunting, upright habit of growth and abnormal production of secondary shoots. They seldom kill plants. Some of these diseases are highly infectious, some are transferred with considerable difficulty, and a few have never been transmitted except by grafting. Many of the diseases of plants caused by virus are spread by sucking insects. Some show an incubation period in the carrier insects. They are most readily transmitted to rapidly growing plants. Plastic cell inclusions are associated with several different mosaic diseases. These intracellular bodies are present in chlorotic tissues, but are not found in the normal green tissues of diseased plants.

STUDIES ON VACCINIA VIRUS. E. H. OPPENHEIMER, Department of Pathology, Johns Hopkins Medical School, Baltimore.

By means of differential centrifugalization, it was determined that the specific gravity of vaccinia virus lies between 1.12 and 1.13. In material obtained by this treatment of ordinary vaccinia lymph, myriads of small granules in masses, pairs and chains were seen by dark-field illumination. The infectivity of the material, as determined by the production of vaccinia lesions on the scarified

cornea of the rabbit, was always associated with the presence of these granules. Purification of the virus was accomplished by centrifugalization and washing in a fluid slightly heavier than the virus.

The virus could not be filtered through any grade of Berkefeld filters. When filtered through paper, wool or chamois, infective filtrates always contained myriads of these granules.

The granules, then, seemed to form an important part of the infective material. To determine the relative importance of the granules and their suspending fluid, Dr. Cracium and I isolated these materials by differential centrifugalization and tried to make each multiply in connection with growing epithelium as Steinhart and Lambert had done with the whole virus.

We removed 0.1 cc. of the top layer of the whole virus after centrifugalization, and added it to 0.9 cc. of sterile Locke solution. The granules were washed thoroughly by pouring off the supernatant fluid and adding fresh Locke solution. This was mixed, again centrifugalized, and the precipitated granules washed twice again in a similar fashion. Before inoculating the granules into the tissue cultures, they were always diluted to their original concentration in sterile Locke solution. The first supernatant fluid was used as the control material. This was not diluted, as it already contained Locke solution. The most satisfactory tissue for culture was embryonic rabbit cornea, grown in homologous heparin plasma. A drop of the diluted washed granules or control material was added to a bit of cornea with a drop of plasma, sealed over deep hollow ground glass slides, and incubated at 38 C. Every three to seven days, the cultures were washed in Locke solution, and put side by side with a new piece of cornea and fresh plasma. In the intervals, that is, every two or three days, the fluid part of the cultures was pipeted off, and a fresh drop of plasma added. About every eight days the presence of the virus was tested by inoculation on a scarified rabbit's cornea. The washed granules and control material used for the series were similarly tested beforehand, the granules always, and the control material never, giving the vaccinia lesion. Whereas the fluid part of the vaccinia virus will not produce a lesion, even after being in contact with growing epithelial cultures, the granules will keep alive for seventy-one days and through nine transfers. They not only live, but actually increase in potency. It was shown further that, as with the virus, living tissue is necessary to the life of the granules.

Another variation was tried to determine whether the granules enter the growing cell or whether they merely remain alive in the plasma and will disappear if the original piece of tissue is removed after being in contact with the new piece for a few days. The cultures were made as above, and transferred to a new bit of cornea after seven days. At this time a camera lucida sketch of the culture was made. The original inoculated piece of tissue was labeled "A," and the fresh piece of cornea to which granules were never added, labeled "B." After seven more days, both "A" and "B" were removed, washed in Locke solution separately, and each added to a new medium of cornea and plasma, thus giving rise to an "A" and "B" subdivision of the same series. After a sufficient length of time, both of these were inoculated separately to test for the presence of the virus. Both series contained live virus, the "B" as well as the "A," showing that it not only is located inside the cell, but that it must, in part, leave the original site of inoculation for the more favorable fresher tissue. The "B" cultures always had a shorter incubation period after inoculation, and gave a larger vaccinia lesion than did the "A" cultures.

Morphologic proof of an increase in the number of granules was not gained from these studies, since they cannot readily be distinguished from other granules

normally seen in tissue cultures. But the experiments differ from others which have been concerned with the cultivation of the virus of vaccinia in that the granules have been washed free from all adhering fluid and then cultivated in increasing potency, while the fluid in which they were formerly suspended does not show power to infect the rabbit's cornea, even after cultivation. They show, at least, that the power of the vaccine to infect an animal is inherent in the granules, even though further analysis of their nature must be deferred.

THE MOUSE AS A TEST OBJECT FOR THE HERPETIC VIRUS. CHARLES E. SIMON,
School of Hygiene, Johns Hopkins University, Baltimore.

SECTION ON COMPARATIVE PATHOLOGY AND IMMUNOLOGY AND
CONTINUATION OF SYMPOSIUM ON FILTRABLE VIRUSES,

RALPH R. MELLON, *Chairman*

A STUDY OF THE SO-CALLED "SPECIFIC FACTOR" (GYE) OF CHICKEN SARCOMA (ROUS). JOHN A. KOLMER, MALCOLM J. HARKINS and JAY F. SCHAMBERG, Research Institute of Cutaneous Medicine, Philadelphia.

A brief summary of Gye's hypothesis of the nature of malignant growths is given, and important technical factors in relation to the preparation of the so-called "specific factor" are described. The results of studies in chicken sarcoma failed to corroborate Gye's work and hypothesis. Evidence indicates that the so-called "specific factor" contains living virus. The virus of sarcoma was not successfully cultivated.

THE VIRUS PROBLEM IN TRANSPLANTABLE TUMORS. J. HOWARD MUELLER, Department of Bacteriology and Immunology, Harvard Medical School, Boston.

Attempts to repeat the experiments published in 1925 by Gye, and from which radically new conclusions as to the etiology of tumors were drawn, have led to a variety of different results, none of which completely confirm Gye, and many of which are totally different. This discussion will deal with his experiments on the Rous chicken sarcoma, from which he prepared a specific factor and a virus, each by itself noninfectious, together causing a tumor in chickens.

His conclusions have been criticized by Harkins, Schamberg, Kolmer and Kast, who found the specific factor controls always infectious when tumors were produced in the mixtures with virus. My own results, and also those of Cutler, are similar to these to the extent that tumors have never been consistently obtained in a mixture of the two factors unless one or the other also gave positive controls.

It is evident that whatever one may believe to be the interpretation of the experiments published by Gye, definite conclusions cannot be reached until the experiments themselves can be repeated by various workers with uniform results. The discordant observations thus far reported must be explained. Three varying factors are involved: the tumor strain, the specific factor and the virus. None of these is of a nature which lends itself to control by simple means, yet obviously each must be controlled before Gye's experimental results can be justly contradicted.

Considerable importance must be placed on the type of controls carried out. Thus we know that there is a marked variation in susceptibility on the part of chickens to injections of the attenuated Rous agent. Our own results demon-

strate clearly that this may be extended even to local variations on the same bird. Two injections of the same material into each of a series of chickens not infrequently lead to the development of one tumor instead of two. If two factors are involved, and controlled at different sites on the same animal, may one be carried by the blood to the site of the second, and produce a false positive? Only by using a fairly large number of chickens for each experiment can one expect to obtain results beyond criticism, and within such a series the observations must be consistent. An occasional result of the type expected, with many others negative, must be held to be fortuitous.

If one may venture to draw conclusions from all the work so far made public, it must be that Gye's experiments have neither been confirmed nor disproved, and further, that it will perhaps be impossible for an individual worker to refute them in a final way because of the uncontrollable factors referred to above. Because of this, negative results cannot, by present methods, signify final refutation. The matter must rest on this basis until Gye himself, or someone with sufficient faith in the results of Gye, or in those of Murphy and of Flu have discovered means of rendering positive results less dependent on uncontrollable accident.

VIRULENT STREPTOCOCCI AND SPORE-FORMING RODS CULTIVATED FROM SO-CALLED HERPETIC AND ENCEPHALITIS VIRUSES. ALICE C. EVANS, Hygienic Laboratory, Washington, D. C.

Five strains of herpes virus and one strain of encephalitis virus received from various laboratories were available for study. By planting emulsions of virus in meat medium, cultures of *Streptococcus* and cultures of spore-forming rods similar to those cultivated from several cases of epidemic encephalitis were obtained from all of the viruses, although ordinary methods of cultivation did not produce growth. Cultures of these forms could also be obtained from filtrates of virus emulsion. Both forms of the organism were virulent for rabbits when inoculated intracerebrally.

ARE CERTAIN OF THE ULTRAVIRUSES, SO-CALLED, FILTRABLE STAGES IN THE LIFE CYCLE OF ORDINARY BACTERIA? RALPH R. MELLON, Highland Hospital, Rochester, N. Y.

In a study published in 1919 I showed conclusively that a filtrable phase of a coccus indistinguishable morphologically from a staphylococcus existed in the blood stream in a case of septicopyemia. This was apparently one of the earliest demonstrations that ordinary bacteria possessed filtrable phases, although Fontes in 1910 made the same claim for *B. tuberculosis*, and in 1916 Almquist confirmed it for *B. typhosus*. Since the discovery of the bacteriophage the proposition has been repeatedly confirmed.

The fact that these filtrable phases of bacteria are usually not cultivable, and are frequently invisible, raises a point of fundamental importance in relation to the so-called filtrable viruses. May many of the latter be a stage in the life history of some visible and commonly recognized micro-organisms?

The question is particularly pertinent when the so-called virus disease is associated with a constant variety of visible micro-organism. The conventional interpretation of the latter as an unrelated secondary invader may thus be seriously impugned. As examples I may mention only the association of *B. proteus* with the virus of typhus, the *B. suispestifer* with the virus of hog cholera and *Streptococcus viridans* with epidemic encephalitis.

The work to be presented today indicates that *B. fusiformis* and related organisms have a filtrable phase in their life history, uncultivable in vitro, but not invisible. Moreover, under the conditions of the experiments it is clear that this phase had an infectivity in guinea-pigs not possessed by the visible phase. The typical lesions and the accompanying bacterial flora have been reproduced in guinea-pigs. The experiments open up considerations of obvious epidemiologic importance. They raise the question whether actual epidemic infections are initiated by the invisible and uncultivable phases of common and perhaps avirulent bacteria.

EXPERIMENTAL IMMUNIZATION IN POLIOMYELITIS. W. L. AYCOCK and J. R. KAGAN, Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston.

Killed virus of poliomyelitis does not possess an immunizing power. Immunization with virus treated with phenol or by drying is successful in only one fourth of the animals used, while about the same proportion of animals become infected from the subcutaneous injection of the "attenuated" virus. Subcutaneous injection of active virus also immunizes, but causes infection in about the same proportion of animals as in the case of "attenuated" virus. It appears that virus subjected to phenol or to drying has little if any less infectivity than active virus when injected subcutaneously.

Active virus injected intracutaneously has caused infection in only one out of twelve animals. Of eleven monkeys receiving intracutaneous inoculations of active virus over periods of from thirty-three to one hundred and forty-seven days, seven resisted from one to three intracerebral inoculations; two resisted one, but were infected by a second intracerebral inoculation. In one of these the incubation period was prolonged. One of the vaccinated animals succumbed to a single intracerebral inoculation, but the incubation period was prolonged.

Blood serums of eight vaccinated monkeys neutralized virus in vitro. Fifteen specimens of serums neutralized the virus, while two specimens partially neutralized it, as was evidenced by prolongation of incubation period. These serums were from animals whose serums had previously neutralized the virus.

In respect to the epidemiology of poliomyelitis these observations lend support to the idea that individuals may become immunized to the virus of poliomyelitis without manifestation of the disease, and that the neutralization of virus by blood serum of persons not known to have passed through an attack of the disease is evidence of immunity resulting from the virus, and not a property of normal serum.

DIABETES MELLITUS: AN EXPERIMENTAL STUDY ON THE ETIOLOGY OF THE DISEASE. PRELIMINARY REPORT. D. H. BERGEY, Department of Hygiene, University of Pennsylvania, Philadelphia, Pa.

Since the etiology of diabetes mellitus has not been traced to either bacterial or protozoal infection, it was believed that the steady increase in the death rate indicated some definite toxic action, and suggested the possibility that a filtrable virus may be the responsible agent. The question was approached by passing the urine of diabetic patients through Berkefeld filters and then inoculating rabbits by administering a dose of about 2 cc. of the filtrate into the blood stream. In about three weeks, glucose appeared in the urine of the rabbits.

On inoculating the filtered urine of diabetic patients into serum broth medium, there is apparent increase of infectivity, since the inoculation of a small dose

of the culture caused the appearance of glucose in the urine in about a week. Subcultures are still infective after cultivation for six months.

The filtered urine of diabetic rabbits, when injected into the circulation of normal rabbits, causes the appearance of glucose in the urine in about a week. The filtered urine of a diabetic patient sealed in glass ampules for over nine months is still infective for rabbits.

GENETIC STUDIES ON RESISTANCE TO BACTERIAL INFECTION. E. ROBERTS and L. E. CARD, University of Illinois.

In 1924, several hundred chicks were inoculated by feeding through pipets a pure culture of *Salmonella pullora*, the causative organism of bacillary white diarrhea, one of the most commonly fatal diseases which affect young chicks. A few were found which apparently resisted infection, and these constituted stock for future breeding.

Chicks from this "resistant stock" in 1925 gave a survival value more than twice as great as that in control chicks. In 1926, 55 per cent of the progeny of the resistant stock survived inoculation, while only 10.1 per cent of the control stock survived. Among progeny of hens selected for one generation 48 per cent survived, while among the progeny of those selected for two generations 60 per cent survived.

In the fall of 1926 the chickens which in the spring tests had above 66 per cent survival among their offspring and those below 45 per cent were put into separate lots. The chicks from the "high lot" gave a survival value of 80.7 per cent, while those from the "low lot" gave 61.0 per cent.

The mortality of chicks from infected hens was not any greater than the mortality of chicks from noninfected hens.

STUDIES OF BACTERIAL ALLERGY WITH THE PNEUMOCOCCUS. HANS ZINSSER and FRANCIS B. GRINNELL, Department of Bacteriology and Immunology, Harvard Medical School, Boston.

Extreme forms of skin reactions are difficult to develop in guinea-pigs by ordinary methods of sensitization with bacteria.

Injections of autolyzed pneumococci into a large series of guinea-pigs, if the autolysates are properly prepared, give occasional violent reactions, with hemorrhage, necrosis and ulceration in about 45 per cent of the old animals, but in practically none of the young animals of 250 to 350 Gm.

These reactions, at first regarded as primarily toxic reactions, have been shown to be allergic by the following facts:

Animals that are negative on first test can invariably be sensitized by repeated injections. Such sensitization can be removed by one or several injections of considerable amounts of the autolysate.

Sensitization can be obtained by a number of different methods, most of them following the ordinary laws of sensitization in general, but it is particularly noticeable that many guinea-pigs can be rapidly sensitized by daily injections in from four to seven days.

The reactions are extraordinarily severe when fully developed, covering areas of an inch square or more, with sagging edema toward the abdomen and destruction of central tissue. They are more violent than the most severe tuberculin reactions obtainable in guinea-pigs. They can be elicited, both as to sensitization and final test, by the products of autolysis and products of bile solution of centrifugalized masses of pneumococci.

It is likely that the responsible antigen is an autolytic product and that bile solution is merely a hastening of autolysis, as suggested by Atkins. It may be, however, that this method of preparing the antigen is merely one of getting an unaltered antigen into solution.

It is suggested that if other bacteria could be similarly autolyzed, similar severe and rapid sensitization might be accomplished with all organisms. This is being studied.

It is also suggested that actual sensitization of the infected body occurs in this way because organisms that cannot be prepared easily *in vitro* in the manner in which this is possible with the pneumococcus are probably subjected to lytic change under the influence of the tissues in lesions. This may explain why reactions with tuberculin, abortin, etc., are best obtained in animals in which living bacteria and changes in tissue around the bacterial foci occur. It suggests an explanation for our success in sensitizing with dead tubercle bacilli when tubercles are formed around them, and would explain our former results in which the action of tissue on tubercle bacilli and tuberculin seemed to be involved in tuberculin reactions.

The speed of sensitization and the suddenness of desensitization, together with the well known and massive autolysis of pneumococci in the human lung during pneumonia, suggests a relationship between sensitization to the autolytic substances and the toxemias and sudden critical desensitization of the patient in pneumonia.

OBSERVATIONS ON THE EPIDEMIOLOGY OF LOBAR PNEUMONIA. M. A. JACOBSON,
Department of Hygiene and Bacteriology, University of Chicago.

The results obtained in this study, in general, confirm the observations made by previous investigators on the occurrence of carriers of the fixed types (I, II and III) of pneumococci; that in lobar pneumonia, the majority of instances of infection are due to organisms belonging to types I and II; that pneumococci of type III are responsible for the smallest percentage of cases of lobar pneumonia; that healthy persons intimately associated with cases of lobar pneumonia may harbor in their mouth secretions pneumococci of the same type as that isolated from the diseased person; and that convalescents from lobar pneumonia may carry for a long time (150 days or more) the type of pneumococcus which was isolated during the infection.

The percentage of fixed type organisms seems to be approximately the same in the white and colored races within a given period.

The data show that the seasonal occurrence of fixed type pneumococci in the throats of normal persons parallels the case rate of lobar pneumonia in Cook County Hospital. In other words, there are a greater number of normal persons showing fixed type organisms in their throats during the pneumonia season than at any other time of the year.

From the evidence presented, the susceptibility of the person seems to be of greater importance in determining an attack of pneumonia than the mere transmission of a so-called virulent variety of pneumococcus.

Type III pneumococci associated with lobar pneumonia are of high electrophoretic potential and of high virulence for white mice and human beings. On the other hand, the same type of organisms (as determined by serologic methods) isolated from normal throats are of low potential and, in a parallel manner, of low virulence for white mice, and, as far as we can ascertain, do not seem to have harmful effects on the human host.

The data indicate that electrophoretic potential measurements are more valuable as indexes of virulence of various strains of pneumococci than tests of virulence with white mice.

THE BACILLUS OF SWINE ERYSIPELAS CAUSING A DISTINCTIVE AND SEVERE FORM OF ERYSIPELOID AMONG FISH HANDLERS. MALCOLM J. HARKINS, Research Institute of Cutaneous Medicine, Philadelphia.

The similarity between lesions of erysipeloid of fish handlers and those of swine erysipelas in man and swine suggested a common bacterial cause. Skin and subcutaneous tissue excised from infected areas revealed an organism having the morphologic, biologic and serologic characteristics of the bacillus of swine erysipelas. Virulence of organisms and serologic reactions in infected individuals were determined.

STUDIES OF THE ETIOLOGY OF THE COMMON COLD. W. C. NOBLE, JR., E. A. FISHER and D. H. BRAINARD, Bacteriological Research Laboratory, Metropolitan Life Insurance Company, New York.

A Comparison of the Flora of the Upper Respiratory Tract in Persons in Health, and with Colds.—In January, 1926, an experimental study was begun of the etiology of the common cold. Cases of acute coryza were selected for study from among the employees of the company who were suffering from the infection, and for comparison, a simultaneous study was made upon a number of healthy volunteer subjects.

In the work herein reported the clinical history was first taken, and then a thorough examination made of the nose and throat. Separate cultures were then taken with straight swabs from the nose, the tonsil and the oropharynx. Cultures from the nose gave for the most part pure, or almost pure cultures, of staphylococci or diphtheroids. In the tonsillar cultures, green and hemolytic streptococci occurred in relatively high incidence. The oropharynx yielded a greater variety of types than the other regions investigated. Without exception, all our patients with acute coryza had symptoms of oropharyngitis and nasopharyngitis. It was therefore decided to discontinue nasal and tonsillar cultures, and to make our study on a single culture from each subject taken from the nasopharynx with a bent swab.

Cultures taken in the winter and early spring from twenty-one healthy persons showed the green streptococci to be the predominating organisms (49 per cent of the entire aerobic flora). Pneumococci comprised 3 per cent, staphylococci 18 per cent, indeterminate streptococci 6 per cent, and hemoglobinophilic bacilli 1 per cent. In cultures from eleven acute cases of coryza the green streptococci constituted 41 per cent, pneumococci 12 per cent, staphylococci 3 per cent, indeterminate streptococci 9 per cent and hemoglobinophilic bacilli 14 per cent. In acute coryza, therefore, the basic normal flora, as represented by the green streptococci and the staphylococci, appear to be decreased relative to the other aerobic types, while the pneumococci, the indeterminate streptococci and the hemoglobinophilic bacilli are relatively increased. The case incidence likewise for the pneumococci and the hemoglobinophilic bacilli was increased in colds. These changes are also brought out in the serial culture studies. In this preliminary study, we did not attempt to classify serologically any of the organisms encountered.

The Nasopharyngeal Flora of Persons in Health and with Colds, Studied by the Serial Culture Method.—The work herein reported was commenced in February, 1926. In March it was suspended to enable us to make a bacteriologic

study of so-called epidemic grip. With the exception of this interruption, the study has so far been carried on for ten months.

Our problem has been to study the changes occurring in the nasopharyngeal flora of healthy persons when colds develop. Cultures have been taken at approximately weekly intervals from eight volunteer subjects, while they have been free from colds, and at more frequent intervals when colds have been contracted. Our results may be summarized in the following manner: 1. There is a definite increase in the numbers of organisms when colds develop (7,600 average for healthy subjects, to 16,000 average in colds). 2. The predominating basic normal types are represented by the gram-positive cocci, especially the green streptococci, hemoglobinophilic bacilli; gram-negative cocci, and diphtheroids constitute other organisms encountered in small percentages in the flora of healthy subjects. 3. There is a relative decrease of the green streptococci as colds develop. There is an increase of the indifferent streptococci, the hemoglobinophilic bacilli and the gram-negative cocci. 4. In certain individual cases of colds, bacterial types new to the subject appear, especially bacilli of the hemoglobinophilic group. These new types may become for the time being, the dominant organisms. Many of them show a tendency to persist for some time after the cold has passed, but in that event their comparative incidence becomes lower.

Nasopharyngeal Flora of Cases of So-Called Epidemic Grip.—In February and March, 1926, there was an extensive outbreak of so-called epidemic grip in New York City. In March a study was made of the nasopharyngeal flora of twenty-seven cases selected from patients reporting for treatment at the company's clinic for employees. The study was not begun until after the peak of the epidemic had passed, but the cases selected were clinically typical for the outbreak.

The results show: (1) that there was an increase in the total number of organisms (an average of 7,800 per case of grip to 17,000 per normal control subject); (2) a comparative incidence of organisms as follows: hemolytic streptococci, 1 per cent; green streptococci, 36 per cent; pneumococci, 12 per cent; staphylococci, 15 per cent; indeterminate streptococci, 10 per cent; hemoglobinophilic bacilli, 8 per cent; gram-negative cocci, 10 per cent; miscellaneous organisms, chiefly diphtheroids, 8 per cent of the total aerobic flora; (3) the predominating organisms were the green streptococci in thirteen cases, pneumococci in five cases, staphylococci in four cases, indeterminate streptococci in two cases, hemoglobinophilic bacilli in one case, *M. catarrhalis* in one case. Meningococci were found in two cases. Thus the outbreak did not show a characteristic bacteriologic picture. Park and Williams reported a case incidence in New York of from 80 per cent to 100 per cent for hemoglobinophilic bacilli for the pandemic of 1918, and Williams, Nevin and Gurley of 92 per cent for the secondary outbreak of 1919-1920. Our average figures for thirty-five normal control subjects show, for the hemoglobinophilic bacilli, a case incidence of 23 per cent; and for our twenty-seven grip cases, of 45 per cent. The outbreak of 1926 is therefore characterized by its relatively much lower case incidence for the hemoglobinophilic group of bacilli.

A STUDY OF THE BIOLOGY OF *B. FUSIFORMIS*. JOSEPHINE S. PRATT, Fifth Avenue Hospital, New York City.

Pure cultures of fusiform bacilli isolated from the throats of man and rabbits were tested for cultural and immunologic reactions and for pathogenicity. They fall into two groups, based on the ability of some strains, the minority, to

ferment saccharose. There is no apparent immunity relationship, the serums of immunized rabbits fixing complement only in the presence of the homologous antigen. Pathogenicity is slight and variable. Fusiform bacilli occur normally in the throats of monkeys and guinea-pigs, but strains from these sources were not studied.

FAILURE OF THE MOUSE TEST TO DEMONSTRATE THE PRESENCE OF TYPE I PNEUMOCOCCUS IN SPUTUM. AN UNUSUAL INSTANCE. RUTH GILBERT and C. K. DAVENPORT, New York State Department of Health, Division of Laboratories and Research, Albany, N. Y.

The use of the mouse method for the determination of the types of pneumococcus present in sputum is so widely recommended that an instance in which this procedure failed to demonstrate the presence of type I in a specimen containing both types I and III seems worthy of note. A specimen was received from a case of lobar pneumonia, on the third day of illness. Avery's culture medium was used, and a mouse was inoculated. Tests with the culture in Avery's medium yielded evidence of the presence of type I pneumococci. On learning these results, the physician administered antipneumococcus serum, and late in the afternoon of the fourth day the patient's temperature, which had reached 105.6 F., began to subside and continued to do so until normal was reached at midnight of the following day. Recovery was rapid and apparently complete. The mouse that had been inoculated was found dead at the end of forty-eight hours. Agglutination and precipitation tests made with material from the peritoneal cavity reacted with type III pneumococcus serum only, and cultures of type III pneumococci were isolated from the peritoneal fluid and heart's blood. In view of these results, another specimen was requested, and a mouse inoculated with it gave similar results with the agglutination and precipitation tests, but colonies of both types I and III were isolated from the heart blood and peritoneal fluid.

STUDIES ON STANDARDIZATION OF ANTIGEN FOR KAHN TEST. R. L. KAHN and NATHAN NAGLE, Michigan Department of Health, Lansing, Mich.

Various lots of powdered beef heart occasionally produce antigens of somewhat different titer and sensitiveness in the Kahn test. This is believed to be due to variations in the amount of lipoids extracted from the beef heart. Antigens may be corrected to a standard titer of 1 + 1.1 (1 cc. antigen + 1.1 cc. physiologic sodium chloride) and to the required sensitiveness by various methods which will be fully described elsewhere. Two of these methods are briefly presented.

If an antigen gives a titer of more than 1 + 1.1, it is diluted with 95 per cent alcohol containing 0.6 per cent cholesterin. To determine how much dilution is required, two 5 cc. amounts of cholesterinized antigen are diluted with 0.75 cc. (15 per cent) and 1.5 cc. (30 per cent) cholesterinized alcohol, respectively. These two diluted antigens are now employed in 1 + 1.1 cc. titers with a half dozen or more weakly positive and a few negative serums, using standard Kahn antigen (obtained at the Michigan Department of Health or on the market) as a control. The diluted antigen, which gives results practically identical with the control antigen, is taken as the new standard, and the entire amount of antigen is accordingly corrected with cholesterinized alcohol. Only two antigens in a large series required dilution with more than 30 per cent cholesterinized alcohol. These two antigens gave titers of 1 + 1.6 and 1 + 1.7, respectively, and they both required 45 per cent dilution with cholesterinized alcohol to bring them to standard requirements. This dilution method may also be employed

with antigens giving titers close to $1 + 1.1$. Occasionally, as little as 10 per cent dilution with cholesterinized alcohol will bring such antigens to the desired sensitiveness.

If an antigen gives a titer of $1 + 0.7$, $1 + 0.8$ or $1 + 0.9$, it is concentrated as follows. Two 5 cc. amounts of the alcoholic extract are evaporated to dryness by means of a revolving fan. The residues are redissolved in amounts of 5 cc. and 10 cc., respectively, of the cholesterinized antigen. The modified antigens are filtered and tested in $1 + 1.1$ titers with weakly positive and negative serums, using the standard antigen as a control similarly to that described above. The antigen which gives results practically the same as the control is taken as the new standard.

Beginners with the Kahn test should procure sufficient standard Kahn antigen to use as a control in standardizing their antigen.

RELATION BETWEEN APPEARANCE OF PRECIPITATE AND POTENCY OF SERUM IN KAHN REACTION. R. L. KAHN, ELIZABETH McDERMOTT and NATHAN NAGLE, Michigan Department of Health, Lansing, Mich.

Precipitation reactions are considered four plus in the "routine" Kahn test when definite flocculi are suspended in clear fluid in each of the three tubes. In such reactions, the bulk of precipitate is approximately four times as great in the first tube, which contains 0.05 cc. of antigen dilution, as in the third tube, which contains 0.0125 cc. of this dilution. Aside from the difference in bulk, precipitates are not always identical in appearance, being more flocculent in some cases than in others. An attempt was made to determine whether there is a direct relation between the appearance of precipitates and the potency of serums. It was found that four plus serums of comparatively weak potency, having from 4 to 80 reacting units as determined by the quantitative Kahn reaction, frequently showed heavy flocculent precipitates, while serums of high potency containing up to 400 or more reacting units, in many cases showed less heavy flocculi. Certain serums showing heavy flocculent precipitates in the first two tubes and much finer flocculi in the third tube were also found to contain comparatively large numbers of reacting units. It is recommended therefore that Kahn reactions in which the flocculation in the routine test is not marked and which are likely to be read, for instance, $+++$, $++++$, $++++$, as well as those which one might read $++++$, $++++$, $+$, should not be averaged to final readings of three plus, but instead, to check such reactions either with a regular quantitative test (standard antigen dilution, 0.01 cc.; serum dilutions, 0.15 cc.) or with a two-tube quantitative test using 1:5 and 1:10 dilutions of serum. A serum giving a positive reaction in one or both of these dilutions, has either twenty or forty reacting units, and should have a final four plus designation.

In the case of weakly positive serums, the first tube of the Kahn test shows little if any precipitation, owing to the inhibitory effect of an excessive amount of antigen, the second tube more precipitation, depending on the potency of the serum, and the third tube, containing the least amount of antigen, the most marked precipitation.

In the quantitative reaction, it is occasionally observed that the undiluted serum shows a finer precipitate than the 1:5 and subsequent serum dilutions. In such instances, the undiluted serum reaction is read positive on the basis of the heavy precipitates shown by the diluted serum.

In reading precipitation tests, it is important to use clear, thin-walled glass tubes which are free from scratches and absolutely clean.

ANAPHYLAXIS: STUDIES V. ALLERGIC RESPONSES OF THE EMBRYONIC CHICKEN HEART. NOBLE P. SHERWOOD, Department of Bacteriology, University of Kansas, Lawrence, Kans.

Studies were made on passive sensitization and reverse anaphylaxis of two or three day old chick embryos. Antiserums were prepared from chickens and rabbits. This necessitated an investigation of the viability of chick embryos at different temperatures, the regularity of the cardiac rhythm and the effect of various kinds and amounts of normal blood serums on the heart rate, rhythm and amplitude. Methods for the study of the embryo either attached to or detached from the yolk were worked out. Lewis' observations on the viability and the effect of temperature on rate and rhythm of the excised heart were confirmed. Locke-Lewis solution was used in the bath. If embryos were selected whose rhythm was regular, and high titered immune serum from chickens added to the bath, they frequently would become apparently sensitive to nontoxic doses of antigen. This was manifested by pauses in diastole and a decrease in rate and apparently in amplitude. As a rule, when the embryo was attached to the yolk, this would develop in two and a half minutes after the addition of the antigen. Normal rhythm could frequently be restored by washing several times. When the same dose of antigen was again added, the only effect observed was occasional slowing. When reversed anaphylaxis was tested for and obtained, the phenomenon would appear in about thirty seconds. A second addition of the same amount of antiserum, after several washings, did not give any response. Negative results in a short series were obtained using antiserum prepared from rabbits. Permeability phenomena apparently exercised considerable influence in both toxic and sensitization phenomena. While reactions of this type may occur under conditions such as described by Lewis, it is thought that the phenomena occurring under proper conditions is an expression of anaphylaxis.

ANAPHYLAXIS: STUDIES VI. PASSIVE SENSITIZATION OF TURTLES AND GUINEA-PIGS USING IMMUNE SERUM FROM CHICKENS AND RABBITS. NOBLE P. SHERWOOD and C. M. DOWNS, Department of Bacteriology, University of Kansas, Lawrence, Kans.

Twenty turtles were injected with high titered immune serum from chickens, and sixteen with immune serum from rabbits. They were all tested, after varying intervals of time, for passive sensitization, using the cardiac response as a criterion. Tracings of both auricular and ventricular behavior were obtained. The antigen was injected intracardially. Tests were made for desensitization and toxic phenomena. A positive reaction is indicated by a decrease in amplitude, more or less slowing and an apparent rise in tone. This is similar to the response obtained by Downs in actively sensitized turtles. Of the turtles receiving high titered immune serum from chickens, six gave positive reactions as compared to one giving a reaction that may be positive out of 16 receiving immune serum from rabbits. The latter needs further confirmation. Observations were made on the shortest and longest time intervals after injection that positive reactions were obtained. Reversed anaphylaxis was apparently demonstrated once.

Twenty-eight young virgin guinea-pigs were injected with antiserum from chickens, and tested for passive sensitization by the Schultz-Dale reaction. All gave negative reactions. A short series tested by the clinical method also gave negative reactions. The latter is in accord with Doerr's results.

A study was made of the cardiac response for guinea-pigs using the technic described for turtles. While some results were apparently positive, the series of both tests and normals was too short to serve as a basis for final conclusions.

ANAPHYLAXIS: STUDIES VII. ACTIVE ANAPHYLAXIS IN TURTLES. C. M. DOWNS,
Department of Bacteriology, University of Kansas, Lawrence, Kans.

A short series of turtles injected several times with mammalian serum into the body cavity were tested for sensitization from three to seven days after the last injection. After pitching, the heart was exposed, the auricle and ventricle attached to a heart lever, and the record made on a smoked drum. After a normal tracing had been made, the antigen, in doses of from 0.3 to 0.7 cc., was injected into the ventricle. If reaction took place, the same amount of antigen was again injected to test for densitization. If a second reaction took place, both reactions were considered. Sixty per cent gave a reaction which was presumably anaphylactic since the second injection of antigen did not cause a change. The positive reaction consisted of a decrease in amplitude of both auricle and ventricle, more marked in the auricle. There was also a rise of tone and slight slowing. The reactions were identical with those observed by Sherwood and Downs in passive anaphylaxis in turtles.

THE RÔLE OF HOMOLOGOUS IMMUNE SERUM IN MICROBIC DISSOCIATION. PHILIP
HADLEY, University of Michigan.

The outstanding characteristics of microbic dissociation are described, and the S, O and R culture types resulting from this reaction are related to certain cyclostages embraced in the bacterial life cycle. The most characteristic and frequently observed differences between the S and the R forms of culture are catalogued. The frequent correlation between S type culture, high virulence and nonphagocytability on the one hand, and, on the other, the correlation between R type culture, nonvirulence and phagocytability, are emphasized. The inciting causes of microbic dissociation are enumerated, and, among these, the stimulus of homologous immune serum is considered in detail. It is pointed out that the action of immune serum cannot commonly be regarded as germicidal, although it is sometimes bacteriolytic. Its chief effect on the culture is to produce a dissociation with the consequent production of the R culture type. It is shown that this has been amply demonstrated *in vitro* in the case of several important pathogenic species, such as *Bact. leproseptica*, the diphtheria bacillus, the pneumococcus and the streptococcus. Certain evidence also suggests that this reaction occurs in the body. Since the fact of microbic dissociation *in vivo* would serve to transform a virulent but nonphagocytatable organism into a nonvirulent but phagocytatable organism, it would appear that the function of the bacteriotropic antibodies may be interpreted as that of producing microbic dissociation *in vivo*. It is suggested that this is in reality the mechanism of the "preparing," opsonic action. Since homologous immune serum is the best inciting agent known in dissociation-provoking power, and since it is clear that such serum is not usually germicidal in marked degree, it is suggested that, in the future, the goal of much therapeutic endeavor will be to combat infections in the body through the use of substances that may possess dissociation-furthering power, even though they may not possess significant germicidal action.

MICROBIC DISSOCIATION AND THE MUTATION MYTH. PHILIP HADLEY, University
of Michigan.

With reference to the systematic aspects of bacteriology, it appears that bacteriologists as a rule have occupied themselves in attempting to perfect schemes of classifications rather than in studying the nature and cause of bacterial variation. When new or unusual forms of culture have arisen, they have usually

been looked on as contaminations, as involution forms or as "mutants." There exists at present among bacteriologists a marked tendency to discover mutants. But the author holds that none of these new forms, even though they manifest some degree of permanence in subculture, justify the use of the term mutation. The reason for this is that the so-called mutants merely represent partially stabilized cyclostages in the life history of the species concerned. It is pointed out that both d'Herelle and Bordet have set up the bacteriophage as an agent endowed with the power of producing mutations from normal cultures, and that the former has expressed the view that all fixed bacterial mutations are produced in this manner. The conclusions cannot hold true, because it is shown that all the bacteriophage can accomplish in this direction is to produce a dissociation of the culture characterized by the formation of new culture types, or cyclostages. All of these new culture forms, however, can be produced, although more slowly, by the culture itself without the intervention of the foreign bacteriophage. The "normal" production of these new forms, may be accelerated by the use of certain "incitants" to dissociation. The author concludes that the bacteriophagic "mutants" of d'Herelle and of Bordet are therefore only partially stabilized stages in the cyclogeny of the species, and that true mutation is at present unknown among the bacteria.

MICROBIC DISSOCIATION IN *B. SUBTILIS*. M. H. SOULE, Hygienic Laboratory, University of Michigan.

When pure strains of *B. subtilis* are cultured in fluid mediums at least two types of organisms can be isolated: the normal or "S" form which is equivalent to that first described by Ehrenberg and later by Cohn, Koch, Klein, Eisenberg, Flügge and Chester, and an "R" or resistant form which varies morphologically and culturally from the classical type and which has not been found adequately described in the literature. In broth the "R" strain produces nonmotile filaments, often attaining a length of 200 microns, which rapidly agglutinate with the formation of a flocculent precipitate. The surface colonies on agar are somewhat irregular and flat, with cuneate surfaces which sparkle by oblique, transmitted light. Microscopically these colonies cannot be differentiated from anthrax. This form does not liquefy gelatin quite so rapidly as the normal type, and the colonies on gelatin are composed of twisted filaments and characterized by edges that are curled under.

Biochemically, the two strains have been found identical in their reactions.

Inoculation of large volumes of fluid medium with the "S" type results in the production of "R" forms in proportion to the quantity of medium and the interval of active growth. Quiescent cultures do not dissociate, and spores fix their own correlated vegetative culture type. Normal forms may be produced by "R" strains by inoculating large volumes of fluid medium, but the percentage of "S" forms obtained from "R" types is much smaller than the percentage of "R" forms produced from "S" strains under the same conditions. The "R" type thus seems to be the stable form. The "S" form is more strongly antigenic. Immune serum has a marked dissociating power on its homologous germ and a stabilizing action on the opposite form. By the incorporation of "S" or "R" immune serums in fluid medium, "R" forms can be obtained from "S" forms, and "S" from "R," respectively.

In addition to pure laboratory cultures, recently isolated strains from hay infusions, air, water and milk have been studied. Both the "R" and "S" forms have been obtained from the foregoing sources. It would seem that the "R" form, being the more stable type, must have been isolated and described before. *B. cereus* as described by Chester may be the "R" form of *B. subtilis*.

FUNCTIONAL AND MORPHOLOGIC CHANGES IN THE LIFE HISTORY OF *DERMACENTROXENUS RICKETTSI* WOLBACH, THE CAUSATIVE AGENT OF ROCKY MOUNTAIN SPOTTED FEVER. R. R. SPENCER, Hygienic Laboratory, Washington, D. C.

Recent studies on the behavior and staining characteristics of the causative agent of Rocky Mountain spotted fever, *Dermacentroxenus rickettsi* Wolbach, in the tissues of ticks and mammalian blood suggest at least three developmental phases in the life cycle of this parasite. These phases are: a dormant or resting stage in hibernating ticks, a virulent, highly fatal phase in nymphal and adult ticks following feeding and the phase in the mammalian host.

THE EVOLUTION OF *B. RADIOBACTER* FROM MIXED CULTURES OF *B.* AND *PSEUDOMONAS RADICICOLA*. THOMAS E. RICHMOND, Ohio State University.

We have been able to show by growing cultures of certain legumes, nodule-forming organisms in mixture (*Bac. radiculicola* and *Ps. radiculicola*) that a third type of organism develops which can be identified as *B. radiobacter*.

B. radiobacter is not nodule-forming as are the parent cultures from which it is derived although it is often mistaken for them. *B. radiobacter* is a non-symbiotic nitrogen fixer, and can be derived experimentally from mixtures of the nodule-forming symbiotic types.

By nitrogen fixation and nodule formation on navy bean roots I have been able to show a great variation in the nitrogen fixing power of different strains of the nodule organisms common to the navy bean plant.

THE OCCURRENCE OF BACTERIOPHAGE AND VARIATION IN *STREPTOCOCCUS* COLONY FORMATION. L. O. DUTTON, Methodist Hospital, Memphis, Tenn.

In a previous report concerning the bacteriophagic activity manifest against the streptococci some data were presented that indicated that certain colony variations from the normal were indicative of "mixed" cultures in the sense of being mixed with phage. By suitably varying the cultural conditions it has been found that many strains of streptococci manifest these colony variations. It is a radical assertion to say that all strains of the streptococci occur as mixed strains. Additional data are offered to substantiate this contention. More detailed study of suitably selected strains of streptococci give evidence of definite transmissible lysis, although it is not usually maintainable through many passages. The colony variations in question have been successfully induced in a normal strain by the addition of filtrates of streptococcus cultures that were thought to contain bacteriophage. Similar colony variations have been observed in strains of organisms other than streptococci which were capable of spontaneous lysis and capable of instituting transmissible lysis. Similar colony variations have been produced experimentally in a normal strain of *B. typhosus* by a definitely known antityphoid phage. It has also been possible to show that attempts at lysis of a mixed strain other than streptococcus show peculiarities of the phenomenon similar to that observed in the studies on streptococci. In view of such evidence it seems necessary to admit other manifestations than transmissible lysis and plaque formation as criteria of the presence of the bacteriophage.

THE RÔLE OF THE BACTERIOPHAGE IN *STREPTOCOCCUS* INFECTIONS. III. AS A FACTOR IN THE RECOVERY FROM INFECTION. L. O. DUTTON, Methodist Hospital, Memphis, Tenn.

Seven cases are reviewed in which the bacteriophage was administered as a therapeutic agent. The result of five recoveries out of this series indicates

that there are possibilities in this direction for the control of streptococcus septicemia.

A review of five cases of streptococcus septicemia in which a therapeutic agent was not used, and in which spontaneous recovery occurred, indicates that the bacteriophage is probably a factor in the recovery of these patients.

A STUDY OF THE TOXICOGENIC PROPERTIES OF SOME STREPTOCOCCI. MARTIN FROBISHER, JR., and J. HOWARD BROWN, Department of Pathology and Bacteriology, Johns Hopkins Medical School, Baltimore.

A review of the literature, correspondence and experimental data shows that strains of streptococci regarded as the etiologic agent in scarlet fever form a heterogeneous group, differing widely among themselves culturally and immunologically. Additional doubt as to the true nature of the etiologic agent of scarlet fever is raised by reports in the older as well as in recent literature of organisms other than streptococci for which there seems to be good evidence of etiologic relationship to scarlet fever.

The evidence on both sides seems conflicting, but also more or less convincing. Acceptance of either view makes it difficult to explain a number of puzzling things which have been observed during the study of scarlet fever.

An hypothesis is suggested which not only appears to reconcile some of the conflicting views, but provides an answer to many questions which have arisen in connection with the problem.

It is suggested that any suitable organism, usually *Streptococcus pyogenes*, may be induced to form scarlet fever toxin under the influence of a certain hypothetical principle, virus or second factor. The streptococci or other organisms found in etiologic relationship to scarlet fever may be supposed, according to this hypothesis, to play a nonspecific part. The unknown second factor is regarded as the specific agent. Neither alone is capable of causing scarlet fever. It is conceivable, according to the idea, that when nontoxicogenic organisms are subjected to the influence of the second factor, they may acquire the power of producing toxin. An organism having the power of causing scarlet fever may previously have been capable of merely maintaining a foothold in the throat or at most causing a localized infection.

In an experimental investigation of this hypothesis, it has been found possible to induce two or three strains of toxicogenic streptococci to acquire temporarily the power of forming toxin of considerable strength and neutralizable with scarlet fever antitoxin.

The method consisted in cultivating the nontoxicogenic strains in sterile Berkefeld "V" filtrates of, or in mixed culture with, the toxicogenic strain and then recovering them. The methods of control have obviated the possibility of impure culture and error due to any of the original toxic filtrate.

It is believed to be indicated that not every cell of a given culture of nontoxicogenic streptococcus will acquire the toxicogenic property. It also seems to be indicated that the factor concerned in the transfer of this property may have moderate resistance to heat.

THE INFLUENCE OF DIPHTHERIA TOXIN ON THE GROWTH OF CERTAIN BACTERIA. J. M. SHERMAN, C. N. STARK and PAULINE W. STARK, Cornell University.

The influence of diphtheria toxin on the growth of bacteria has been tested. The toxin was added to beef infusion broth so that the resulting medium contained 30 M.L.D. per cc. A number of bacteria tested did not show a detrimental effect of the toxin on their growth, but an exception was found in the case of a culture carried in this laboratory under the name of *Bacillus cereus*. With

B. cereus, a small but definite retardation of growth in the presence of toxin has been repeatedly observed.

While it cannot be definitely stated that the factor contained in the diphtheria toxin preparation which is toxic to *B. cereus* is the same factor which is toxic to higher animals, certain observations support that view: 1. Heating entirely destroys the factor which is inhibitory. 2. The toxic factor for *B. cereus* is apparently inactivated when sufficient antitoxin is added to neutralize the diphtheria toxin.

These observations suggest interesting possibilities. If the toxin is in fact toxic to some bacteria, it may be possible for such organisms to respond by the production of a specific antitoxin. As yet we do not have any evidence supporting this view. Since it is known that toxins may be destroyed by proteolytic enzymes, it is conceivable that the growth of some bacteria in the presence of diphtheria toxin would result in the destruction of its toxicity without impairing its antigenic properties.

EFFICIENT PRODUCTION OF AGGLUTININS. CHARLES A. BEHRENS, Purdue University.

It was desirable to determine whether or not high titer production depended on the amount of the antigen injected at one time or on the frequency of stimulation of the body cells of the animal by the inoculation of small amounts of foreign protein. Consequently special attention was directed to dosage, total amount injected and the frequency of the inoculations of the agglutino-gen necessary to produce a satisfactory agglutinin formation.

It was shown that small doses of an agglutino-gen inoculated not too frequently over a short period of time produced agglutinins of a higher titer than larger amounts extended over the same or longer period of time.

Similar experiments were conducted using various precipitinogens. In the production of precipitins the advantage of small dosage over larger amounts was further conclusively demonstrated.

The development of hemolysins by these methods proved to be unsatisfactory except in the case of sheep red blood cells.

OBSERVATIONS ON THE SOLUBLE ANTIGENS OF BACTERIUM ENTERITIDIS. SARA E. BRANHAM and ELEANOR M. HUMPHREYS, Department of Hygiene and Bacteriology, University of Chicago.

Toxic, bacteria-free filtrates of cultures of *Bact. enteritidis* grown in a protein-free medium, when injected into rabbits, stimulated the production of agglutinins, precipitins, complement-fixing antibodies and possibly antitoxins.

When these filtrates were concentrated by evaporation in vacuo, the products so obtained were better antigens than emulsions of *Bact. enteritidis*.

Protein could not be demonstrated, even in the dry residue obtained when such a filtrate was evaporated to dryness. When this dry residue was dialyzed, an opalescent fluid and a gray precipitate were obtained. Protein could not be demonstrated in either of these fractions. The precipitate contained carbohydrate. Further concentration of the opalescent fluid dialysate gave a liquid that showed faintly positive reactions for tryptophan and histidine and a faintly positive ninhydrin reaction. Protein must therefore have been present. The vanillin and diazo reactions will detect tryptophan and histidine, respectively, in dilutions of 1:1,000,000. Calculated on the basis of these tests, amounts of filtrate containing as little as from 0.000,003 to 0.000,04 Gm. of protein led to definite antibody production. Whether or not so small an amount of protein can be held exclusively responsible for this antibody production, the possibility that

it may do so should be borne in mind in interpreting results obtained with apparently protein-free materials.

SYNTHETIC MEDIUMS FOR DIFFERENTIATION OF THE TYPHOID-PARATYPHOID GROUP OF BACTERIA. LUTHER THOMPSON, Mayo Clinic, Rochester, Minn.

Synthetic mediums consisting of various amino-acids and inorganic sources of nitrogen may be used in identifying typhoid and paratyphoid organisms. In this work twenty-eight typhoid, ten paratyphoid A, and eleven paratyphoid B strains were used. With valine as a source of nitrogen all strains of paratyphoid B grew, 10 per cent of paratyphoid A strains grew and only 3 per cent of typhoid strains. If glutamic acid is used as a source of nitrogen the percentages of positive growth are as follows: paratyphoid B, 100 per cent, paratyphoid A, 80 per cent; typhoid, 18 per cent.

Synthetic mediums could be used to advantage as an additional cultural test, especially in cases of atypical agglutination in the paratyphoids.

BACTERIA IN THE SUPPOSEDLY STERILE MECONIUM. SEVERANCE BURRAGE, UNIVERSITY OF COLORADO.

Examination of nearly 100 meconium samples obtained from infants just born and before suckling, showed the presence of bacteria in about 38 per cent of the samples. In the positive cases—and none was called positive unless the organisms were numerous—*Bacterium coli* was found in pure culture in about 50 per cent. The other positive cases usually contained a staphylococcus in pure culture, but there were a few mixed cultures containing two or three species.

The infants from whom the material was obtained were all born of healthy mothers, and with normal delivery.

The paper describes the method of obtaining material, the care used to eliminate all outside contamination and the methods of making the cultures.

ANTIVENIN SPECIFICITY. AFRANIO DO AMARAL, Antivenin Institute of America, Glenolden, Pa.

Specificity of antitoxins or antibacterial serums in regard to their respective antigenic principles or germs seems to be well established.

To the majority of immunologists this seems also to be the case in regard to the antivenomous serums or antivenins. A few serum therapeutists, however, even among the most modern ones, apparently do not concur with those views. The Pasteur Institute of Paris, for instance, still states that its antivenomous serum, which is known to be specific for the cobra, is effective against various venoms, such as that of rattlesnakes, scorpions and tarantulas.

The statement induced me to investigate the question in a general way by using several types of venom in cross experiments with a corresponding number of antivenins. The venoms used were from: *Crotalus terrificus*, *C. atrox*, and *C. adamanteus*, *Bothrops jararaca*, *B. jararacussu*, and *B. alternata*, *Micrurus corallinus* and *M. frontalis* (snakes, and *Tityus bahiensis* (scorpion).

The following conclusions may be drawn from these experiments: 1. Specificity is a general phenomenon among antitoxins. 2. "Group specificity" may also be found that will eventually justify the administration of a particular antivenin against the venom of species congeneric or closely related to that against which the antivenin is specific. 3. Application of antineurotoxic or anti-hemolytic serums or antivenins, against all neurotoxic or hemolytic venoms, however different zoologically the species from which the latter proceed may be, appears to be untenable.

AMERICAN ASSOCIATION FOR ADVANCEMENT OF
SCIENCE, SECTION N (MEDICAL SCIENCES)RUFUS I. COLE, *Chairman*; A. J. GOLDFARB, *Secretary**Philadelphia, Dec. 29, 1926*

GROWTH IN HEALTH AND DISEASE

1. SOME ASPECTS OF SEXUAL DIFFERENCE IN PRENATAL GROWTH AND DEATH.

OSCAR RIDDLE. (Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, Long Island, N. Y.)

Relatively few facts are now available in any of the three main groups of facts which are here considered: (1) exact quantitative data for the prenatal growth of organs or systems of organs; (2) the range of causes of prenatal death; (3) the rôle of sex in either prenatal growth or death. The purpose of the paper is to consider three groups of recently developed facts which seem to involve all of these three questions, and which seem to indicate that in placental mammals (and indeed in still other animals) the prenatal environment is not equivalent for male and female fetuses, but shows some elements prejudicial to the male.

The male fetus is forced to accept the female environment of the mother's uterus. Present evidence shows that at least one allegedly sex-specific hormone passes from the mother's blood into that of the fetus, and exerts an influence antagonistic to masculine development. This alcohol soluble hormone (obtainable from ovary, placenta, etc.) is probably responsible for the accentuated and essentially abnormal prenatal growth of the prostate, mammae, suprarenals and Müllerian duct system of the male. Other alleged regressive or degenerative changes in the testes, penis, epididymis and Cowper's glands are noted. These phenomena of prenatal growth, degeneration and antagonism may provide unequal chances for the male and female fetus to live.

Of a different nature are the serologic changes arising in the male fetus and in the mother when a male fetus develops, according to recent reports from the Frauenklinik in Halle (Sellheim, Lüttge, Merz). From results obtained by a modification of the Abderhalden reaction it is found that the mother's blood reacts against a testis substrate when a boy is carried in utero. Thus, this blood test of the mother is declarative of the sex of the fetus, and of the presence of an unusual component in the maternal blood. This "anti-testis" body is considered capable of entering the blood of the male fetus. Further, in the blood of the male fetus some product (apparently not a hormone) is also found. Against the female fetus such a body is not formed. In this second way, therefore, the male and female probably have unlike prenatal environment, and in both cases the difference seems adverse to the male.

A third point is concerned with new facts indicating that in certain respects the nutritive requirements (vitamine B, protein, and metabolic) for growth and for health are unequal in male and female. Since these are apparently greater in the male a higher death rate of males should result in that fraction of mothers whose nutritional status, in respect to these particular elements, is near the border line. It is true that many of these particular data have been obtained on postnatal stages, but there can be little doubt of their applicability to fetal life. Fleish and Hess, and Hartwell, conclude that male rats require for their normal growth relatively more vitamine B than do females; and

that male rats are first affected by restricted amounts of this vitamine. Slonaker and Card found that when parent rats were suddenly changed to a restricted diet the proportion of male offspring was reduced. Hartwell further concludes that the male rat is more susceptible to the quality of the protein components of its food. The probable higher metabolism of the male fetus would also probably tend, like the other conditions just noted, to bring a higher prenatal death rate on the male side.

It is an observed fact that the prenatal death rate in the human and some other mammals is notably higher in the males, and that the earlier the death the higher is the proportion of dead males. The conclusion currently drawn from these facts by physicians, geneticists and others is that the "male is inherently weaker than the female"—bearing more lethal factors, etc., than does the female. This conclusion can only be drawn on the assumption that intra-uterine life provides an entirely equivalent environment for male and female. The foregoing discussion makes it certain that such equivalence does not exist. Further, all of the three aspects of difference described are adverse to the prenatal growth and health of the male. The fact that for some post-natal years the human male death rate continues relatively high may mean only that adverse conditions of prenatal life—the sensitive period of highest growth increment and differentiation—can injure without causing immediate death.

2. ON THE DISTRIBUTION OF DIFFERENCES IN VITALITY AMONG INDIVIDUALS.

RAYMOND PEARL. (From the Institute for Biological Research of the Johns Hopkins University.)

This paper defines vitality, in a general sense, to mean the degree of intensity of vital actions. Since this is a quantitative concept it is necessary to proceed at once to the consideration of its measurement. In the broadest terms such measurement will always finally depend on the rate of energy transformation of the organism, within defined limits in respect of space and time.

Inherent vitality is defined as that part of the total vitality which is dependent solely on the innate organic pattern or organization (in the Aristotelian sense) of the individual.

By the measurement of the inherent vitality in suitably devised experiments on duration of life in the fruit-fly *Drosophila*, and on the growth of seedlings of the cantaloupe *Cucumis*, results are obtained which are offered as a novel contribution to the direct study of the organic action pattern of individuals.

The results show:

1. The distribution of individual differences in inherent vitality is of the same form as that of differences of total vitality, when vitality is measured by total duration of life under conditions (a) of starvation and (b) of normal feeding, temperature and other environmental conditions being the same in the two cases.

2. The distribution of individual differences in inherent vitality is of the same form when measured as the integrated time variable total duration of life, or as the differential time variable, rate of growth.

3. The distribution of individual differences in inherent vitality is of the same form in such widely different organisms as *Drosophila melanogaster* and *Cucumis melo*.

These facts would seem to indicate that in the concept of inherent vitality here developed a matter of real biologic importance is being dealt with.

3. SOME PROBLEMS OF CELLULAR PHYSIOLOGY. W. J. V. OSTERHOUT. (The Rockefeller Institute for Medical Research.)

Two tendencies have been particularly noticeable in the recent progress of biology. The first is greater attention paid to the underlying causes on which all vital activities depend. There is a deepening conviction that in pure and applied biology, especially in medicine and agriculture, the paths of progress that have been choked by the growth of empiricism must be cleared by new methods of attack and these must be discovered by quantitative studies of the fundamental activities of the cell.

The second is the growing belief that these fundamental activities are largely controlled by the permeability of the protoplasm, by which is meant its power to exclude certain substances while admitting others more or less freely. Hence, the study of permeability has acquired especial importance not only in theoretical respects but also in many practical problems, such as the absorption of food, the application of drugs, and the physiology and pathology of secretion and excretion, especially in relation to such organs as the ductless glands and the kidney.

The first step in this study is to find out what substances enter the protoplasm and how rapidly they penetrate. The best procedure would be to place the cell in a solution and to analyze the cell contents to determine what substances have penetrated, but this has proved unsatisfactory in practice, because the cell contents could not be extracted without alteration. In consequence resort has been had to indirect methods. These, however, have produced so much confusion that it is felt that the only possible way out was to find a method of determining the actual penetration of substances by direct analysis of the contents of the cell, avoiding the difficulties which have hitherto made this procedure so unsatisfactory. It has turned out, fortunately, that this can be done by employing large multinucleate cells whose sap can be obtained without the alterations which always occur when sap is extracted from cells of ordinary size.

The cells employed have been chiefly those of the marine alga, *Valonia* and the fresh water *Nitella*.

These studies indicate that there is little or not any penetration of salts into the cell in the form of ions.

It is found that the cell sap has a different composition from the outside solution and, in the case of *Valonia*, the sap proves to be toxic when applied to the outside of the cell.

One striking feature is that certain substances, such as potassium, are found in much higher concentration inside the cell than outside. This may be explained by assuming that potassium enters as potassium hydrate, and is changed in the sap to potassium bicarbonate, and that bicarbonate ions are exchanged for the chlorine ions of the external solution, so that potassium chloride accumulates in the sap. This process will go on if the production of carbon dioxide inside the cell keeps the sap more acid than the external solution until a point is reached at which the product of potassium and hydroxyl ions inside is equal to that outside.

This assumption would make both growth and the accumulation of salts dependent on the production of carbon dioxide, which is in harmony with the fact that the most rapidly growing cells are those which produce the most carbon dioxide.

4. DIFFERENTIAL DEVELOPMENT IN CONDITIONS OF THYROID DEFICIENCY. **FREDERICK S. HAMMETT.** (The Wistar Institute of Anatomy and Biology, Philadelphia.)

An analysis of the relative growth retardation of the several organs, with respect to that of the body weight, of albino rats of both sexes deprived of the thyroid gland at 23, 30, 50, 65, 75 and 100 days of age, and allowed to grow until 150 days old, shows them to be classifiable under three headings: i. e., resistant, sensitive and special. The eyeballs, brain, spinal cord, humerus and femur, uterus and ovary before puberty, and lungs show relatively greater or the same resistance as the body as a whole to the growth retarding influence of the conditions brought about by removal of the thyroid. The heart, submaxillary glands, liver, kidneys, spleen, uterus and ovary after puberty, suprarenals and pancreas show a greater sensitivity, while the thymus, hypophysis, testis and epididymis give specialized reactions.

The basis of the difference between the resistant and the sensitive organs lies in the ratio of the apportionment of the total metabolism between the processes of maintenance and the processes of growth. The first phase comprises largely the working over and elaboration of materials for use outside of the particular organ in question. The second phase consists largely in the incorporation into the organ substance of materials relatively inert and but sluggishly available, in the metabolic sense, for the rest of the body.

Growth is the expression of the fact that more material is available than is needed for maintenance under the given conditions. It is a truism that functional needs dominate growth processes under adverse conditions. Hence, when the amount of available materials is decreased by the lowering of the metabolic level due to thyroid removal, it is but natural that those organs which normally have the higher maintenance-growth ratio should be more retarded in their growth, than those in which the maintenance processes are less in proportion to the total metabolism or energy exchange. The group of sensitive organs belongs in the first category; the group of resistant in the second. Contributory is the relative degree of stability or availability for metabolic purposes of the substances incorporated into the organs, which gives rise to increase in weight, the expression of growth. Because of these differences in ratio of metabolic distribution, it is clear that the relative degree of normal growth capacity expressed in weight, cannot be the index of the relative degree of growth retardation under conditions of thyroid deficiency.

Of the specialized responses, that of the thymus is expressed as a marked sensitivity with great loss of weight. This is attributable to the exquisite sensitivity of the organ to adverse conditions. Its cause is unknown. The hypophysis responds by an acceleration instead of a retardation of growth. This is due to, and is evidence of, a specific relation to thyroid activity, the nature of which is, as yet, inexplicable. The testis is noteworthy in that, though it is an organ of great activity, it is highly resistant to the growth inhibiting influences. This is because the type of activity is a growth and not a maintenance activity. The growth of the testis is largely a matter of increase in cell number. This type of growth is relatively more resistant to thyroid deficiency, than is growth by increase in cell mass. Thus the greater resistance of the testis is explained. The epididymis is similarly highly resistant. The basis of the reaction probably lies in the functional relationship of the organ to the testis as a storage organ for spermatozoa.

The pubertal adjustment introduces a new factor which markedly affects the character and degree of growth reaction of the organs to thyroid deficiency.

The influence of this change reverberates throughout the entire organism, tremendously increasing the sensitivity to the glandular defect.

The implications of these observations are several. They show that growth retardation in conditions of thyroid deficiency is not a simple proportionate affair, but that modification of the relative sizes of the organs is produced which results in shifts in bodily proportions. These, of course result in shifts in relative functional intensities, with their consequent influences on developmental processes.

The thyroid is the most variable organ in the body, responding by changes in size and functional activity to changes in both external and internal environment. It is therefore not going far from the path of scientific rectitude to suggest that long continued modifications of thyroid activity in one direction, brought about by consistent change in environment or habit, voluntary or enforced, may be a cause of species differentiation within a genus, or at least of variety production within a species. If the inheritance of acquired characteristics is possible, there is here available a mechanism by which this possibility could be realized. For it would be absurd to deny the possibility that such profound alteration of differential development as is shown in these animals, with the consequent alteration of relative functional intensity, should not have a modifying influence on the germ plasm. To set this apart as unreactive would be specious.

Upward of 700 unmated male and female albino rats provided the data for this investigation. They were all of the same Experimental Colony Stock of the Wistar Institute, the descendants of two pairs. They were kept under like conditions of diet and environment. All were healthy and free from vermin. The tests were litter mates of the controls.

SOME BIOLOGICAL ASPECTS OF MEDICAL PROBLEMS

1. THE NEEDS OF MEDICAL ENTOMOLOGY. L. O. HOWARD. (Chief Bureau of Entomology, U. S. Department of Agriculture.)

The speaker showed that knowledge of the transmission of disease by insects has increased so rapidly since the initial discoveries by Manson and Ross that no one has had the opportunity to survey the field carefully and to plan future work comprehensively and systematically. This field obviously has a great future. The work so far has been done by isolated persons without the best facilities, and the possibilities abundantly justify the careful training of a large body of skilled workers who shall be given abundant financial support.

There are many problems still unsolved that relate to the insects already known to carry disease to man or animals. These problems should be attacked at once.

There are many insects closely related to these forms, that have disease-bearing possibilities. There are whole groups of such insects the intimate biology of which is but little understood; and study of these should be urged and amply supported. Every feature of their biology should be exploited. The men to do this should be trained in the study of insects. Acquaintance with vertebrate pathology is also desirable, but not absolutely necessary. The control of the insect carrier or potential carrier is the aim of the work, and hence the importance of the trained economic entomologist.

The speaker urged the necessity for a centralized foundation in which a general scheme can be elaborated for a systematic covering of the whole field of possibilities in this direction, which should have enough funds to finance all investigations that bear on the subject.

2. MYCOLOGY IN RELATION TO HUMAN PATHOLOGY. C. L. SHEAR. (Plant Pathology, U. S. Department of Agriculture.)

The constantly increasing number of microfungi which are being found associated with lesions and other pathologic conditions in man, and which have in a considerable number of cases been found to bear a causal relation to such troubles, emphasizes the need for much more exact knowledge of the identity, complete life history and relationship of these organisms. Such information should help to solve some of the problems connected with the origin of infection, the dissemination of the parasites and methods of prevention and cure.

About 100,000 fungi have been named. Many of them are both polymorphic and pleomorphic in character, that is, the same species frequently exhibits different varieties and spore stages in its life cycle, as well as physiologic forms, which in the case of parasites vary in their degrees of parasitism. These facts would seem to indicate the desirability, if not the necessity of cooperation between the pathologist, who has to deal with the microparasitic diseases of man and the expert mycologist, who is thoroughly familiar with the special groups of organisms involved, and the latest information and technic necessary to supply the information needed by the medical investigators. It would seem obvious that the medical pathologist could scarcely be expected to have the training, experience and time necessary to solve these mycological problems for himself.

The multitude of so-called species already named and imperfectly described by medical men needs to be studied carefully and compared with the still greater number of related, nonparasitic forms, in order to determine their synonymy and taxonomic status.

Such names as *Monilia*, *Oidium*, *Sporotrichum*, etc., are frequently applied in entirely different ways by the mycologist and pathologist. This is leading to a dual usage of the names and a different taxonomic grouping, which results in confusion rather than in the advancement of the knowledge. Many of the fungi with which the medical man deals are known to belong to common groups, most of which are not parasites, for example, *Penicillium*, *Aspergillus*, *Mucor*, *Cospora* and *Trichothecium*. Others, however, such as those referred to *Trichophyton*, *Achorion* and *Microsporon*, among the so-called *Dermatomyces* are much more difficult to determine, on account of their unsatisfactory development and failure to sporulate normally in culture. In fact, the more strictly parasitic the organism, the more difficult it is to grow and reproduce normally in pure culture. Lack of cooperation and coordination in this field also leads to great confusion in the terminology and nomenclature. In order to meet the needs of the present situation and to promote the best interests and advancement of both mycology and human pathology, a thoroughly specially trained and experienced mycologist should be connected with each large medical research institution, with the necessary assistants and equipment, to make thorough and detailed studies of these organisms. Greater attention should also be paid to the collection and preservation of typical cultures of all the fungi isolated from man and other animals, and such cultures should be made available for distribution in various culture supply laboratories, in order that they may be available to mycologists and pathologists for comparative study.

3. DENGUE. J. F. SILER. (Medical Corps, U. S. Army.)

A review was presented of the experiments showing that dengue fever is caused by a filtrable virus transmitted by *Aedes aegypti* and not by *Culex*.

4. HUMAN TYPES IN RELATION TO MEDICINE. R. BENNETT BEAN. (School of Anatomy, University of Virginia.)

Four human types have been traced in the literature from the Greeks to the present. The four humors of the Hippocratic school, yellow bile, phlegm, black bile and blood, were related to the four elements of Aristotle, fire, water, earth and air, respectively. The more recent classification of four types in Europe by Chaillou and MacAuliffe, the cerebral, digestive, muscular and respiratory, may conform somewhat to the respective types of the Greeks in the order given above, but attempt is not made to show any relationship.

The cerebral type has a large round head and the other parts of the body are spare; the type has increased with civilization, but it is the rarest of the three types.

The digestive type has huge jaws and lower face, a ponderous abdomen and long arms and legs, with a reach much greater than the stature. The digestive type has been shown by Henckel to have a susceptibility to manic-depressive insanity, with only a slight incidence of dementia praecox. The digestive type is only a little less rare than the cerebral.

The respiratory type has a large middle face and a nasal, large, long thorax, and the other parts of the body are slender and gracefully proportioned. They represent the Raphael and Leonardo de Vinci types. They comprise next to the largest element in the population.

The muscular type makes up the largest proportion of the population. They are rectangular in face, the upper, lower and middle parts are equal, the nose is somewhat rectangular, the neck is broad and muscular, the shoulders are broad and horizontal, the hips are wide, the extremities are long and the whole body is strongly muscular.

There are mixed types of the possible combinations of all four of the primary types, and some persons are not to be classed as any type. Bauer found 43.3 per cent of pure types, 56.7 of mixed types and 8.5 of doubtful types among 2,000 patients.

The muscular-digestive type of person is sometimes a veritable giant, the wrestler type of Bauer, the weight men and heavyweights. Bauer found three types among 4,328 German athletes and students; wrestler, gymnast and pentathlon. The wrestler is large, the gymnast has short extremities and the pentathlon is slender, with long extremities.

Kretschmer and Henckel find three types that exist throughout Europe which they call asthenic, muscular and digestive. Other types are given: the apoplectic, phthisic and asthenic of Stiller; the megalosplanchnic and micro-splanchnic of Viola; the sport types of Theoris, of Kohlrausch and of Philostratus Flavius; the gallbladder, gastric ulcer and others of Draper; the lateral and linear of Stockard; the herbivorous and carnivorous of Treves; the broad back and narrow back of Goldthwait; the stocky and slender of Kraus; the hypersthenic and hyposthenic of Mills; the heavy and slender of Davenport; and a host of others recent and remote. The brachyskèle and macroskèle of Manouvrier and Godin come nearer to any standardization than any of the others. These two are the same as the mesomorph and hypermorph of Bean. To these should be added the hypomorph in any other population than that of Europeans.

The extreme and abnormal forms of the mesomorph and hypermorph may be called mesodermopath and epitheliopath, respectively, because the former is susceptible to diseases of the tissues derived from the mesoderm, and the latter to diseases derived from the ectoderm and entoderm.

5. HORMONE CONTROL OF CYCLIC GROWTH AND FUNCTION OF THE FEMALE GENITAL ORGANS. EDGAR ALLEN. (Department of Anatomy, University of Missouri School of Medicine.)

Recent experiments (the early work on rodents was done in collaboration with E. A. Doisy and others) have demonstrated that ovarian follicles containing growing ova are the principal source of the internal secretion which controls the growth and function of the female genital organs. Injections of extracts of follicular contents seem to be an adequate substitute for the internal secretion of the ovaries in the absence of pregnancy. The active substance has been extracted from hen, pig, horse, cow and human ovaries and has been injected into mice, rats and monkeys (assisted by a grant from the Committee for Research on Sex Problems of the National Research Council). Thus, experimental sexual cycles have been induced after removal of the ovaries in rodents and primates.

The action of this hormone is fundamentally one of inducing growth in the genital organs including the mammary glands. Growth reactions from injections into spayed animals have been equivalent to the maximum occurring in the normal animals under ovarian influences. Decrease in amount or absence of hormone due to cessation of injections is followed by a wave of regression in the genital organs. In the lower animals this is accompanied by leukocytic infiltration, in primates by menstrual hemorrhage. Thus a single hormone may be all that is involved in the necessary mechanism of the nonpregnant sexual cycle.

The only source of this hormone other than the ovaries is the placenta. Extracts of placenta prepared by the same method act in a similar way. The continuous availability of this secretion during the course of pregnancy is largely responsible for growth of the uterus and mammary glands and for the absence of menstruation during gestation.

Several biologic products firms are preparing this hormone and standardizing it according to this reaction in "rat units." Early results from clinical tests (in collaboration with J. P. Pratt) indicate that it may prove to be a useful therapeutic agent in the treatment of reproductive disorders.

NEW YORK PATHOLOGICAL SOCIETY

Anniversary Meeting, Jan. 13, 1927

DAVID MARINE, *Presiding*

COMPARATIVE STUDIES OF THE SUGAR, LACTIC ACID AND ACID BASE BALANCE OF THE BLOOD AND CEREBRO-SPINAL FLUID. MARJORIE R. MATTICE, THERESA GARCIA and JOHN A. KILLIAN. (From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.)

Using Clausen's method, from 8 to 15 mg. per hundred cubic centimeters was obtained as the normal for blood lactic acid, and from 80 to 90 per cent of this for spinal fluid. In meningitis the sugar was negative or very low, with a rise in the lactic acid which was insufficient to account for all of the sugar. In epilepsy the sugar of the blood and spinal fluid rose immediately after the seizure; the lactic acid increased and ran high long after the seizure, whereas muscular contractions gave a sharp rise and fall. Many epileptic patients showed a higher lactic acid content in the spinal fluid than in the

blood. For acid base balance studies, the blood was collected under oil and the spinal fluid over mercury (Shohl's device), and Cullen's method used at 38 C. The reaction of the blood and spinal fluid ran parallel and it appeared that the spinal fluid maintained its acid base balance within physiologic limits, as did the blood. In meningitis the drop in p_H cannot be due to lactic acid alone but also to the production of other acid factors. The results would seem to indicate that the composition of the spinal fluid is dependent on that of the blood, and differs markedly only when pathologic changes are evident in contiguous tissues.

DISCUSSION

DAVID MARINE: If the sugar in the spinal fluid is increased by the administration of sugar intravenously, does that influence the lactic acid content?

JOHN A. KILLIAN: If sugar is given intravenously, it involves an increase in the sugar of the spinal fluid, which, however, lags behind that of the blood. The maximum is seen in the blood probably within one hour after the sugar is given, whereas the maximum in the spinal fluid is seen about three or four hours after. I do not have any direct evidence of a change in the spinal fluid due to the administration of sugar. In a child who received a glucose infusion as a therapeutic agent, there was an increase in the lactic acid in the blood, as would be expected, and there was also an increase in the lactic acid in the spinal fluid, which could be explained only as the result of the glucose.

THE VERNES TEST FOR TUBERCULOSIS. ADELAIDE B. BAYLIS, Membre correspondant de l'Institut Prophylactique, Paris. (From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.)

On previous occasions there have been presented before this society communications dealing with the Vernes flocculation test for syphilis, its technic, the apparatus employed and the results of comparative observations of simultaneous Wassermann and Vernes tests on the same specimens of serums.

Without in any way curtailing their valuable syphilologic service the Institut Prophylactique in Paris has entered a new field of usefulness. Dr. Vernes, for the last four years, has been applying the same principles in the development of a satisfactory serologic aid for the determination of tuberculous infection.

In June, 1926, the Institut Prophylactique published a fourth fascicule (Travaux et Publications de l'Institut Prophylactique, Paris, A. Melone et Fils, 1926) devoted to the researches on the serology of tuberculosis and it was my privilege to be in their laboratories last summer for a period of time and to work with this most recent test.

Description (Baylis, Shepler and MacNeal: The Vernes Flocculation Test as an Accessory Serological Guide in the Combat Against Syphilis, *Am. J. Syph.* 10:298, 1926) of the Vernes, Bricq and Yvon photomètre has been given elsewhere so it is unnecessary to repeat it. It might, however, be helpful to review briefly the phenomena on which the tests are based. To quote Dr. Vernes (Sero-measure de la tuberculose—The Serological Measure of Tuberculosis, *Am. Rev. Tuberc.*, to be published) in an article to appear soon, "The phenomena of flocculation in syphilis and tuberculosis prove that this specific serological reaction is dependent upon the physical state (molecular concentration, number and dimension of the elements in suspension etc.). . . . When one combines human blood serum with a reagent capable of producing a precipitate the turbidity obtained obeys a law which is rather curious. In taking the same amount of human blood serum in a series of small test tubes and from the first to the final tube adding the reagent in a progressive curve, the turbidity

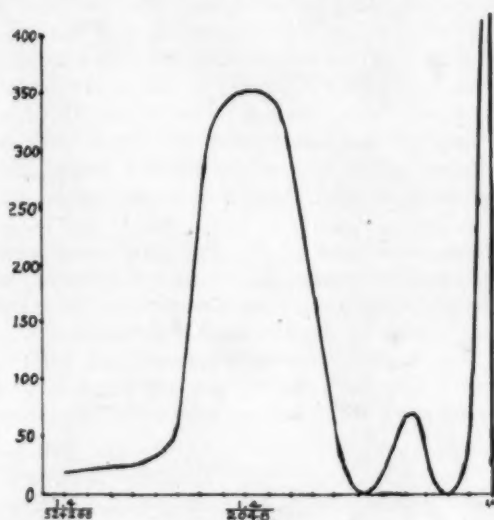
observed from one end to the other of the series of tubes does not increase nor diminish in a regular progressive manner. On the contrary there are waves and the position of the fall and rise sometimes close together and sometimes at a distance, varying with the nature of the reagent employed."

If in a series of twenty test tubes a constant amount of serum is diluted with a solution of sulphate of nickel as illustrated

Volume of solution of sulphate	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	
	524.288	262.144	131.072	65.536	32.768	16.384	8.192	4.096		
of nickel in cc.	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4
	2.048	1.024	512	256	128	64	32	16	8	4

(Volume of unheated serum, 0.2 cc. in each tube.)

the sinusoidal curve obtained will appear as shown in the chart.



Sinusoidal Curve

Applying this phenomenon and employing different reagents, especially among the phenols, it was found that resorcin ($C_6H_4(OH)_2$) produced the most delicate results, and in consequence it has been the reagent so far employed by Vernes. Various concentrations of this chemical substance were tried, and a solution of a 1.25 per cent was determined on. Experiments were performed on inactivation of serums, temperatures ranging from 37 C. to 55 C. being used, and the most satisfactory results were obtained with unheated serums. At the same time incubation temperatures were investigated, and 20 C. was selected as being the one most adapted to the desired constant results. A final series of tests was made on the proportions of serum and reagent in which the amount of the reagent used would give the earliest indications of an infection, without reactions in the normal zone, and an equal proportion of reagent and serum was found to yield the most favorable results.

This brief summary will suffice at present for the history of the researches carried out by the Institut Prophylactique. Much could be written on the subject, and many personal observations made in the laboratories might be recorded, but time does not permit it.

The blood used in the test is obtained by the usual technic of vein puncture, the quantity required being approximately 15 cc. The tube containing the blood is stoppered and placed in the refrigerator over night to allow for concentration of clot and the separation of clear serum, it being of the utmost importance in this test to have the serum exceptionally clear and transparent.

The following morning the serum is decanted and freed from cells by high speed centrifugalization. Two tubes are set up for each patient and 0.6 cc. of the clear serum is placed in each of the tubes. A preliminary, or control reading, is then made in the photomètre of Vernes, Bricq and Yvon, and the figures are recorded. To obtain these readings, 0.6 cc. of the resorcin antigen is added to the serum and the optic density obtained immediately before proceeding to the second tube.

The tubes are then stoppered and incubated in air or water at a temperature of 20 C. At the end of four hours a second reading is made and the difference between the optic density of the incubated mixture and the control or preliminary reading is obtained. This difference is due to the reaction taking place during the period of incubation, and indicates the amount of infection and the differentiation between normal and tuberculous serum. For interpretation of the results the original article should be consulted, but in general, it may be said that readings above 30 are regarded as signifying an active tuberculous process.

This test is simple, easily carried out and possesses the advantages of the elimination of the hemolytic system, the use of a nonspecific, well known, pure chemical substance as a reagent and the recording of the results in numerical form, in which slight variations may be quickly detected.

For the last few months I have employed this test; because of the limited observations I have been able to make, it would be premature to draw any definite conclusions, but the results promise to be interesting.

DISCUSSION

FLORENCE SABIN (by invitation): I am exceedingly interested in this work of Miss Baylis. From the present trend of the experiments on tuberculosis it is clear that the chemical side of the work is becoming more and more important, and it seemed to me as I listened to this paper that possibly one could now start to find out the nature of the substances that give this test. Are they substances from the cells, or are they produced by the bacilli? Possibly they are less likely to be the substances from the bacilli, but they could be more easily tested. Protein and fat fractions are now being produced from tubercle bacilli by Prof. Treat B. Johnson, and if Miss Baylis were to test the blood of an animal after the injection of these fractions she could determine whether they had any effect on the flocculation test or not. On the other hand, tuberculous tissue injected into a normal animal might give results worth comparing with the tests from the chemical fractions.

PAUL KLEMPERER: Were there any differences between the quantitative tests for tuberculosis in children and adults?

MISS BAYLIS: I do not think that has been entered into. I have experimented with serum from children and from adults, and they seem to give the

same figures in flocculation, depending on the degree of infection as estimated by their clinical symptoms.

JAMES EWING: Have you a definite idea as to what substance enters into this reaction?

MISS BAYLIS: No, I have not. I do not know what the substance causing the reaction is, but I do know that a definite reaction is obtained in tuberculous subjects, and it is not obtained in normal people. With an increase in the infection there is an increase in flocculation. As the patients show clinical improvement, the flocculation drops.

LOUIS GROSS: In what percentage of cases of tuberculosis verified clinically and by laboratory tests do you obtain a positive reaction?

MISS BAYLIS: As I remarked, my own observations have been so limited that it is difficult to answer this question from the data, but from the work done in Vernes' laboratory, apparently the remarkable result of 100 per cent is obtained. I know in my limited work with the exception of one case in which there was an error in technic, I have never so far obtained a normal figure in any tuberculous patient. Of course, in regard to some of the patients who have had pneumonia and from whom it has been impossible to obtain specimens to inoculate into guinea-pigs, I could not tell whether they had tuberculosis in combination with the pneumonia or not, but I rather suspected that they had.

LOUIS GROSS: May I ask about the specificity of the test? Is there any other condition which will give a positive reaction?

MISS BAYLIS: There are three zones. Vernes considers the readings of the flocculation from 0 to 15 normal, or rather the inactive zone. The readings from 15 to 30 are the indefinite zone in which the septicemias, cancer and chancre may give a reading. They will not give a constant reading in this zone, while certain degrees of tuberculosis will always give a constant reading, so that the other conditions can be eliminated by a repeated test. Any test above 30 is a definite reaction for tuberculosis. The intermediate zones can be eliminated by repeated tests, and in all cases it is advisable to make repeated tests on patients.

MAX LEDERER: Has the test been used on normal and tuberculous spinal, pleural and peritoneal fluids?

MISS BAYLIS: That work is being carried on, and it seems to run in the same zone and in the same manner. Vernes has had one or two remarkable cases of diagnoses by the test on spinal fluid.

THE RELATION OF THE OPTIC MENINGES TO CYSTS OF THE ORBIT. LOUISE H. MEEKER. (From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.)

An interesting case in the service of Dr. Martin Cohen forms the basis for this study. A tumor removed from the orbit was designated a lymphangioma on gross examination. A brief study of the microscopic sections seemed to confirm the opinion. Further study of later sections caused me to change the diagnosis and finally to investigate the previously reported primary lymphangiomas of the orbit, particularly those found within the muscle cone.

REPORT OF CASES

CASE 1.—*History*.—In a colored boy, aged 4 years, the right eye began to protrude and become painful after a blow. The Wassermann test was positive,

but anti-syphilitic treatment was not beneficial. The exophthalmos increased steadily and a diagnosis of retrobulbar tumor was made. Krönlein's operation was performed five weeks after the injury. At operation, a cystic mass was found behind the eyeball and within the muscle cone. This tumor measured 35 by 20 by 16 mm. It was fairly well encapsulated, and although adherent to its surroundings, was removed without great difficulty. The surface was covered by thick fibrous bands between which there were thinner portions cystic in character.

Microscopic Examination.—The spaces were filled with coagulated albumin or blood. That the blood was due to the trauma of operation was indicated by the fact that the red blood cells were perfectly preserved. Blood clots or old blood pigment deposits were not present. New growth of lymph vessels, lymph follicles and muscle bundles were not found at any point.

One cavity contained a membrane made up of large endothelial cells, many of which were filled with melanin granules, proved by special staining. That this membrane represented the arachnoid membrane in a colored patient seemed inescapable. When the significance of this observation was understood the diagnosis of lymphangioma was doubted. The roentgen-ray examination, as well as the normal development of the eye, ruled out the meninges of the brain as a probable source. It seemed that the spaces filled with blood and lymph were multilocular sacculations of the meninges of the optic nerve.

CASE 2.—This case of orbital cyst in which operation was performed by Dr. Cohen, seemed enlightening. The exophthalmos was found at operation to be caused by a cystic mass lying between the bulb and the nasal wall anteriorly, and outside the muscle cone. Roentgen-ray examination had revealed a much enlarged optic foramen. It was plain that the cyst in this case was a meningo-encephalocele formed by extension from the brain and its meninges.

My first case so closely resembled the histologic description and photomicrographs of a "lymphangioma" of the orbit recently reported that I began a critical examination of all tumors included under that designation.

Thirteen cases reported in the literature as primary orbital lymphangioma may be divided according to location. Eight lie outside the muscle cone and five within the muscle cone. Although the descriptions are meager, all the tumors outside the muscle cone can be identified either as meningoceles or as extensions of lid lymphangiomata.

The five tumors within the muscle cone were of greatest interest. Radiographic information was lacking in every case. All were encapsulated and three were free in the muscle cone but slightly adherent to the optic nerve. These three could easily be classified as possible meningoceles cut off from their primary pedicle. Knowledge of the orbital fissures, however, was not available. Two so-called lymphangiomata of the orbit remain for special consideration. One of these, reported by Kahn, was not primary in the orbit. The remaining case was that of Wintersteiner, and I believe that Wintersteiner was dealing with two defects, both originating at the same time before birth, and in an area of the brain meninges adjacent to and including the optic stalk. The anterior lid tumor conformed perfectly to meningoceles appearing in the lids. The cystic mass encircling the optic nerve corresponded to an anomaly seen in many eyes. To these anatomic vagaries an inflammation has been added, after the manner described by Sick, or the inflammation has preceded the anomaly, if one follows Spring's conception of meningocele formation. Spring maintains that an initial cystic pachymeningitis occurs in all cases. The cysts, then, that have been described are filled with cerebrospinal fluid, to which

blood is added occasionally by accident. Smooth muscle bundles may be found in the septums, but they do not indicate lymphangioma for it is a striking fact that all the descriptions emphasizing the presence of smooth muscle bundles in the septums are of tumors situated anteriorly in the orbit, a region abundantly supplied by smooth muscle, as demonstrated by Hesser in the fascia about the eyeball in man.

My own case can be explained somewhat as follows. The eye was normal, indicating normal development beyond the time of the closure of the fetal cleft in the primary optic vesicle and stalk, and beyond the fusion of the pia and filling in of the nerve stem. This was at about the fortieth day. Between this date and the laying down of the bony orbit something occurred to alter the orderly formation of the arachnoid and dural sheaths of the optic nerve. This possibility is strengthened by the evidence of congenital syphilis. What rôle, if any, injury may have played in the later formation of the cyst is not clear. Bergmeister's case reported in 1913 is considered notable, because in a microphthalmic child in whom optic development was arrested at the stage of the primary optic vesicle, bilateral cysts of the orbit were found. He was able to demonstrate that these cysts were simply the distended optic nerve sheaths. Subsequent writers refer to his report as something new in the development of orbital cysts, meaning that the recognition of the rôle played by the optic nerve sheaths dates from Bergmeister's observation. My case is the first cystic tumor within the muscle cone of the orbit to be described since the appearance of his paper.

Before summing up it is necessary to emphasize that the designation "lymphangioma" should apply only to true neoplasms formed of, or tending to produce, lymph vessels. Lymphatics have not been demonstrated in the orbit of man, except those relating to the lids and about the lacrymal gland. There is ample anatomic and embryologic basis for the explanation of the occurrence of cysts in this region. My case is a combination of embryonal defect with inflammation and ectasia of the optic meninges.

SUMMARY

1. Primary lymphangiomas of the orbit have not been proved to exist. The evidence is too meager and other possibilities have not been sufficiently investigated.

2. The optic nerve meninges bear the same relation to multilocular cysts of the orbit within the muscle cone as do the meninges of the brain to cysts outside the muscle cone.

3. I believe that a descriptive term, such as "multilocular cysts of the optic nerve meninges," to describe such cases as mine, is preferable to the common practice of including them erroneously among lymphangiomas.

4. The term "meningocele" may be applied whenever hernia of the arachnoid can be proved.

5. My case is the only one recorded within the muscle cone in which the arachnoid has been found or identified.

DISCUSSION

JAMES EWING: Dr. Meeker has done a good service in collecting the information on what would appear to the general pathologist to be a rare condition. As for rare conditions, it all depends on one's experience and point of view. To the ophthalmologist these are probably rather frequent occurrences,

and the number of authors which Dr. Meeker had to cite shows that after all rarity does not apply really to these conditions. I agree with her that the condition hardly deserves the term lymphangioma, but that it may not have some neoplastic quality would be difficult to assert. They seem to me more or less comparable to some of the cavernous hemangiomas which are recognized not as true tumors, and yet are classed among the neoplasms, because they have certain neoplastic characteristics. I should like to ask Dr. Meeker if any of the tumors she has seen or read about in the literature were associated with the so-called myxoglioma of the optic nerve.

DAVID MARINE: I would like to ask if this condition has occurred bilaterally, inasmuch as Dr. Meeker thinks it may result from embryonal developmental defects.

LOUISE H. MEEKER: In reply to Dr. Ewing, myxoglioma is rarely, if ever, associated with these cases. Professor Verhoeff has written the final word on myxogliomas of the optic nerve. They are primary tumors of the optic nerve trunk and only involve the sheaths secondarily.

In answer to Dr. Marine, the condition in which arachnoid has been identified has not occurred often enough to discuss, but the case which helped to confirm my opinion, that of Bergmeister, was one of cysts of the optic nerve sheaths in both eyes, both eyes being microphthalmic.

TRUE HERMAPHRODITISM. REPORT OF A CASE. BORIS KWARTIN and JOSEPH HYAMS. (From the Department of the Laboratories and the Department of Urology, New York Post-Graduate Medical School and Hospital.)

We had the opportunity to observe a case of true hermaphroditism in our mortuary. The rarity of the condition and the fact that a heated controversy still centers around the existence or nonexistence of true hermaphroditism urged us to report this case, which, we must emphasize, is offered only as an addition to the cases hitherto reported, without going into abstract theoretical discussion of the nature and pathogenesis of the curious condition.

REPORT OF A CASE

Alexander W., aged 24, colored, considered a male, was admitted to the Metropolitan Hospital on Nov. 18, 1924, suffering with advanced pulmonary tuberculosis. The clinical notes procured through the courtesy of the authorities of the Metropolitan Hospital read as follows: "Patient is a well-developed, well-nourished male of 23 years with female physical characteristics. He has no hair on face but has well-developed female breasts. He has rounded hips; the distribution of his pubic hair approaches the female type."

He died on Dec. 24, 1924, the cause of death being given as advanced chronic pulmonary tuberculosis in a pseudohermaphrodite.

The body came to our mortuary for anatomic dissection. The abstracts of our notes follow:

"A young adult, moderately nourished, apparently male individual, approximately 25 years old. Length of cadaver is 5 feet 7 inches (167.5 cm.). The face is rounded, does not show any evidence of having been shaved; chin and upper lip are similarly beardless. Adam's apple is slightly prominent but not as markedly as in a normal male. The torso is characteristically feminine, shoulders are rounded, breasts are well developed, the nipple shows a distinct areola mamillata. There is no hair on the chest. The pelvis is broad, approaching the female type; the hips are wide; the pubic hair is of female distribution.

Pelvic Measurements

Interspinous (anterior superior) distance.....	22.5 cm.
Intertrochanteric distance	29.5 cm.
Interischial distance	11.0 cm.
Distance from sacro-iliac synchondrosis to symphysis pubis	12.5 cm.
Circumference of pelvis.....	39.0 cm.

"The penis is 5 cm. long, with normal external form. The glans is of a proportionate size to that of the penis. However, at the tip of the glans there is no urethral opening. On the under surface, 2 cm. from the tip of the penis a small hypospadiac meatus 5 mm. wide is seen, into which a catheter can be introduced, leading into the bladder. Scrotum is of moderate size but it is empty. In the right inguinal canal a distinct cord is palpable which is connected with an almond-shaped, moderately firm mass, 2.5 by 2 cm., apparently testicle. Left side is devoid of any masses or cords.

"Both lungs show advanced caseous tuberculosis. Other thoracic and abdominal organs show nothing unusual. In the pelvis there is a small rudimentary uterus with a moderately tortuous tube and a small fibrotic ovary on the left side. On section no corpora lutea or follicular cysts are seen. No tube or ovary can be found on the right side. After removing the pelvic organs en masse with the external genitalia the following features are observed: A wide, penile urethra with two corpora cavernosa. On its posterior wall, 48 mm. from the meatus, there is an opening which leads into a vaultlike structure identified as vagina. Into the vagina a narrow cervix protrudes with a lumen which can be followed into the fundus of the uterus.

"In the right inguinal canal the vas deferens and the veins appear of normal configuration. The cord leads to the testicular mass situated at the level of the vaginal opening into the urethra which measures 28 by 20 by 8 mm. It is of oval shape, moderately firm in consistency with a circular indentation at its upper pole. On section the upper cap-shaped portion, which is firm, striated, pale pink in color, appears to differ in structure from the larger, lower portion, which appears soft and granular with numerous fine connective tissue septa. No prostate or seminal vesicles are seen.

"Microscopic examination of vaginal wall, cervix, uterus and left tube show atrophic lining membrane, while the ovary contains numerous corpora fibrosa, suggesting a senile organ.

"The supposed testicular mass shows an upper portion with characteristic ovarian stroma, 5 or 6 primary Graafian follicles in each section and numerous corpora fibrosa. It suggests a combination of an infantile and a senile atrophic ovary. The lower portion is composed of numerous winding tubules with thick hyaline basement membrane, which support two or three indefinite rows of somewhat cuboidal or polygonal cells. These cells are uniform in appearance, having clear, pale cytoplasm and fairly large, deeply-staining nuclei. Occasional intertubular nests of cells with slightly deeper staining cytoplasm and small nuclei are seen. In spite of the postmortem changes shown by these cells their location and the interpretation of the tubules as seminiferous tubules suggest their being considered interstitial cells of Leydig. The tubules themselves are similar in appearance to those found in the cryptorchic and atrophic testis."

We, therefore, interpret this organ as an ovotestis and our case as one of true hermaphroditism.

COMMENT AND SUMMARY

Hermaphroditism is the mixture of opposite germinal and accessory sexual characteristics. We do not agree with such extremists as Kermanner, Ahlfeld and others who claim that both sex glands must be present in mature form in order to justify the classification of a person as a true hermaphrodite. We accept Pick's and Sauerbeck's contention that the organospecific, gonadal character of the sex glands should be demonstrated: i. e., that the morphology of the organs should be convincing even if they are in an atrophic or rudimentary state. We incline to view the primordial sex gland as endowed with a common bisexual anlage and believe that true hermaphroditism is the expression of an atavistic formative hindrance with the lack of disappearance of one sex gland. The hypoplastic condition of both elements may be explained, according to Halban, hypothetically, as follows: The impulse for the development of the sexual system as a whole, which usually is concentrated on one system, in the case of hermaphroditism is required to act on two systems, with the disastrous result that it becomes insufficient to force either of them to the normal degree of development.

The criteria of true hermaphroditism as established by Sauerbeck and Pick are:

1. In all cases of true hermaphroditism there is a mixture of external male and female sexual characteristics.
2. In most cases of undoubted true hermaphroditism an ovotestis is found on one side or the other.
3. In most cases the testicular portion of the ovotestis is larger but atrophic, the picture resembling that found in cryptorchism.
4. The ovarian portion is usually smaller in size, but more highly differentiated. Primary, primitive and mature Graafian follicles, corpora lutea and corpora fibrosa are recognized.

Applying these criteria to an extensive study of the pertinent literature we found that cases of undoubted human true hermaphroditism have been reported by the following authors: Salén, Simon-Garré, Uffreduzzi, Photakis, Briaux, Lacassagne and Lagoutte, Polano, Schauerte-Berblinger, Burden-Masson, Young and Meyer. To this series we add our case.

The cases of Gudernatsch, Kleinknecht, Sand, Reifferscheid and Reverdin are considered as probable.

We reject the cases of Baldwin, Moots, Losert, Schneider and Pettayel as either incompletely presented or not convincing.

DISCUSSION

DAVID P. SEECOF: Were the other endocrine glands examined, especially the suprarenal and pituitary?

LOUIS GROSS: During life did this patient have catamenia suggestive of menstrual cycles? Today it is relatively simple to test for the so-called female sex hormone in the blood. I do not suppose that this patient's blood was so examined. It is an accurate method of determining whether the patient is predominantly female or not, but I would like to know clinically whether there were such cycles.

F. H. DIETERICH: Did Dr. Kwartin mention whether any spermatozoa were seen?

CHARLES NORRIS: I imagine that these conditions are much more frequent than is supposed. Dr. Burdick at Bellevue Hospital had a case of a woman without any external genital anomalies who had a small tumor in the inguinal region, and my recollection is that there was a perfectly well formed testicle. The cases are not examined sufficiently to tell just how frequent the condition is. They used to be considered rare, and yet they are really much more common than was thought.

JOSEPH HYAMS: The person apparently was admitted to the hospital as a male and was kept on the male side, but the body was accepted as female in our mortuary, and it was only after a rather careful examination that the male characteristics were shown. The important thing from the clinical side is that most of these cases present two conditions. One, of course, is hypospadias, the operation for which does not entail much difficulty, and the other is undescended testicle. It means that the surgeon must be particularly careful in the operation because we find there is an atypical blood supply, and the organ itself is typically a testicle. In this instance, it was only by dissecting away the tunica albuginea that one could see the structure which seemed to have the appearance of an ovary with the tube. Of course, under what circumstances it presents itself is important, as the medico-legal aspects of operation on such a gonad could be disastrous to the patient.

BORIS KWARTIN: In regard to the first question, the body showed advanced postmortem changes, and the microscopic examination of the suprarenals was therefore of little value. The gross and microscopic appearance did not indicate any marked hypertrophy.

In regard to the question of catamenia, the clinical information is meager. Data could not be obtained of the patient's previous life, because he did not have any relatives.

With regard to the question of spermatozoa, the authors have never been able to find mature spermatozoa. The fact that the testicle is hypoplastic is accepted by most authors. Usually the ovary does present a higher development at one side or the other, shown clinically in the form of catamenia.

THE SPLENIC LOBULE.* WARD J. MACNEAL (From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.)

Kyber, Hoyer and, later, Mall, have recognized a lobular unit in the spleen, but they are not precisely in accord nor is the lobule clearly defined by any of them. By employing perfusion and fixation of the spleen in the distended state, it has been possible to elucidate the circulation in the spleen pulp and to arrive at a clearer conception of the lobular unit as determined by the arrangement of the finer vascular channels.

The unit of spleen structure, to which it is proposed to apply the term lobule, consists of splenic follicle or malpighian corpuscle, its important surrounding marginal zone and the irregularly radiating pulp cords surrounding this, together with the included vascular channels. The lobule is limited at its periphery by a zone of larger venous sinuses into which the venous sinuses of contiguous lobules drain. The splenic lobule is spheroidal in man, but more irregular in form in the rabbit. Compound lobules occur in both species and are relatively more abundant in the rabbit. The lobule, as here designated, corresponds in a sense to the conception of Kyber. It corresponds less well to that of Mall.

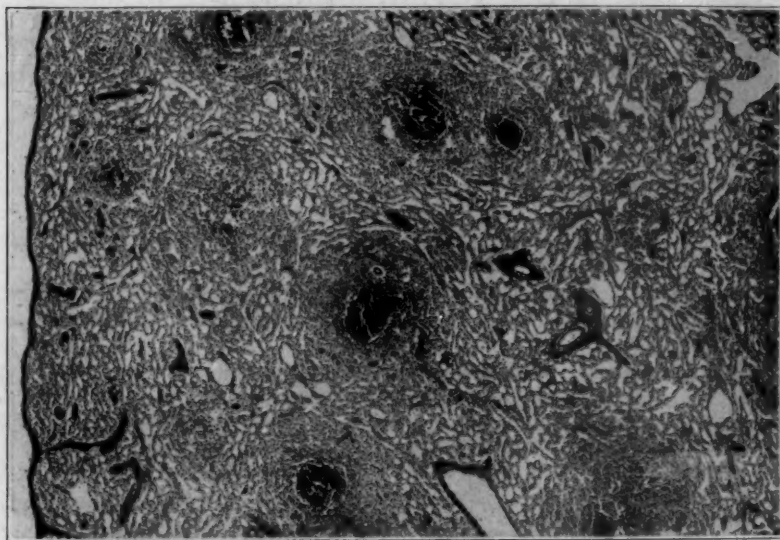
* The full paper will appear in the Anniversary Volume dedicated to Alfred Scott Warthin, published by George Wahr, Ann Arbor, 1927.

DISCUSSION

FLORENCE SABIN (by invitation): I am naturally interested in Dr. MacNeal's presentation of the splenic lobule. The demonstration of the three zones in the lobule and the showing that the periphery of the lobule in the depth of the organ is marked by the veins of the splenic pulp clears up the subject most convincingly. What is the meaning of the spaces seen in the margin of the follicles whenever the spleen is fixed during distention? I have often seen them before, and there were a few in Dr. MacNeal's slides.

ALFRED PLAUT: In sections of spleen stained for fat the peripheral zone occasionally contains a much larger amount of fat.

WARD MACNEAL: I wish first to apologize for presenting a purely anatomic topic before the New York Pathological Society. However, when one comes



Photomicrograph of section of human spleen, perfused and fixed in the distended state immediately after splenectomy; near the center of the figure is an intralobular arteriole with lymph follicle below it, marking the center of a lobule; the periphery of the lobule is marked by distended venous sinuses; above and slightly to the right is a compound lobule in which two follicles are associated without distended sinuses between them; after a little study the entire area of the figure can be resolved into its constituent lobules.

to study the pathologic condition of the spleen, it is obvious that a finer knowledge of its anatomy is needed. The whole question is fundamental to the pathologic condition of the spleen. When this work was begun, I felt that I was doing something I should have done as a first year medical student. In studying histology I was tremendously puzzled over the spleen and never had any clear idea of it. The conception of a unit lobule of the spleen with the distribution of the blood vessels in it, and the peculiar way in which the blood passes through the lobule seems to me to give a fundamental anatomy on which one can base some ideas concerning function. In the recent literature of the spleen

one sees everywhere the recognition of the importance of the marginal zone. It is perfectly obvious that this must be the region in which active function takes place in the spleen. It seems to me that the studies made so far finally prove that in the marginal zone there is the discharge of the red blood corpuscles of the patient himself into the intercellular spaces between the reticular cells of the spleen, so that the red blood cells come into direct contact with these cells. It is in this region that the erythrocytes are most actively phagocyted.

In regard to the question asked by Dr. Sabin, the behavior of the marginal zone after perfusion has been a problem to every one who has studied this question. It is an exceedingly difficult question. In almost every distended spleen tears and ruptures will be found; hence, in my preparations I am willing to recognize the presence of artefacts in which the cells are torn, but I am also willing to maintain that there are portions of the specimen in which such an explanation is not adequate, and the elements are merely stretched without rupture. Distention in the marginal zone is far less than that obtained at the periphery of the lobule, where the sinuses are larger. The illustrations shown in some of the lantern slides will appear in a forthcoming article in the *Journal of Pathology and Bacteriology*.

The question of fat in the marginal zone does not seem to me to require further discussion. It is evident that this is the extremely active region of the splenic lobule. If injury has taken place in the spleen, it may first be made evident in this zone.

REPORT OF AMERICAN SOCIETY OF PARASITOLOGISTS

Second Annual Meeting, Philadelphia, Dec. 28, 29 and 30, 1926

About seventy-five members of the society were present out of a total membership of 392. A feature of the program was an interesting and splendidly illustrated address on "Piroplasms" by Prof. G. H. F. Nuttall of Cambridge University. Dr. Nuttall gave a review of the life cycles of a number of representatives of this group and ended with a plea to American parasitologists to devote more attention to the many unsolved problems connected with these organisms. The retiring presidential address by Dr. C. W. Stiles was devoted to a timely discussion of certain problems in zoological nomenclature.

A total of forty-five papers were listed on the program of the society, of which thirty-four were read by the authors and eleven presented by title. Of the forty-five papers, seventeen concerned protozoology, twenty-seven helminthology and one medical entomology. About one fourth of the papers were on the systematic-morphologic phases of the subject. Most of the others were experimental in nature, dealing with immunity, life cycle relations or the economic and medical phases of the subject. A further analysis of titles and abstracts show that twenty-three papers dealt either directly or indirectly with human parasitology, that eight had to do with parasites of animals of economic importance to man and that fourteen might be called strictly of zoological interest. Some of the points of particular interest to medical men will be mentioned briefly.

W. H. Taliaferro and his co-workers reported a series of 1,605 tests with thirty-seven different antigens, made on 535 persons in Honduras, in the attempt to find a precipitin test for human malaria. Using an antigen prepared from heavily infected placentas they found a high correlation between the positives detected by the precipitin test and those found in thick smear. They conclude

that their results indicate that a precipitin test can be devised for malaria. L. R. Cleveland reported the cultivation, for more than a year, in tap water, of a tritrichomonas obtained from human feces.

In a paper on the host parasite relations between the dog and its hookworm, C. A. Herrick showed an increased resistance to the parasite in older dogs. He also found that dogs harboring hookworms are more resistant to infection than those that were nonparasitized or those that had been freed from the hookworms before reinfection.

J. E. Ackert and his co-workers reported that young chicks fed with a diet deficient in the fat soluble vitamin A are less resistant to the establishment and growth of the common chicken nematode *Ascaridia lineata* than chicks of the same age fed with a diet in which this element is present in sufficient quantity. The discussion of this paper emphasized the importance of studies on the relation of the diet of the host to its resistance to parasitic infection.

H. W. Brown gave the results of some studies on *Ascaris lumbricoides* in Panama, in which he demonstrated that eggs on the floors of the huts were an important source of infection. He also showed that ascaris and trichuris eggs develop under natural conditions on the soil much more rapidly than has previously been supposed.

In a paper by W. E. Dove and G. F. White, studies on the etiology of creeping eruption in man in the southern United States were reported, which demonstrated that this condition can be produced by the penetration and migration of the infective larvae of the dog and cat hookworm *Ancylostoma braziliense*.

M. S. MacDougall showed that the increases of the sugar content of the blood of canaries produced by the feeding of sugar rendered it a more favorable habitat for the malarial organisms.

A higher incidence of intestinal protozoa in a group of seventy-one persons was obtained by C. F. Craig and J. H. St. John by the culture method than by either sedimentation or direct microscopic examination. They found that a medium composed of seven parts of physiologic sodium chloride solution and one part of inactivated human blood serum was suitable for a continued cultivation of *Endamoeba histolytica* over an indefinite period of time.

R. W. Hegner reported the excystation of the cysts of the human *Giardia* in the intestine of the rat. F. L. Soper suggested, from the finding of a *Ankylostoma-Necator* ratio of 13 to 1 in semicivilized Indians in the Paraguayan Chaco, that *Ankylostoma duodenale* may have been introduced into the Western Hemisphere before the discovery of America by Columbus.

It is not possible even to mention the numerous other interesting observations reported. Abstracts of all the papers on the program will be published in the March number of the *Journal of Parasitology*.

The following officers were elected for 1927: president, R. P. Strong; vice-president, Edwin Linton; new members of the council for a four year period J. H. St. John and F. D. Barker.

W. W. CORT, Secretary.

Book Reviews

PROTOZOOLOGY, A MANUAL FOR MEDICAL MEN, VETERINARIANS AND ZOOLOGISTS.
By C. M. WENYON. In 2 volumes, with 565 illustrations and 20 colored plates. Price, \$25. New York: William Wood & Co., 1926.

The author of this manual was formerly protozoologist to the London School of Tropical Medicine and is now director-in-chief of the Wellcome Bureau of Scientific Research. He has long been an investigator of the protozoa and his published researches date back to 1906. Leishmanias, trypanosomes and intestinal protozoa have furnished the principal material for his investigations. The present work is in two volumes and covers the entire phylum protozoa. Volume I of 778 pages, contains part 1, General Description of the Protozoa and a portion of part II, Systematic Description of the Protozoa with Special Reference to Parasitic and Coprozoic Forms. Volume II, of 795 pages, completes part 2 and includes part 3, Spirochaetes, part 4, Methods of Investigation and Rules of Nomenclature, part 5, Blood Parasites of Vertebrates and Trypanosomidae of Invertebrates, part 6, References to Literature and the Index.

In part 1 (150 pp.) is given a general account of the morphology, nucleus and nuclear division, encystment, reproduction and life-histories of the protozoa. Of special interest are sections on immunity in protozoal infections (13 pp.), in which are discussed natural immunity, recovery from infections, acquired immunity and the mechanism of immunity, and the action of drugs in protozoal infections (2 pp.). The author considers the question of the unicellular or noncellular nature of the protozoa and decides in favor of the former. He stresses the point, with which I thoroughly agree, that "to understand properly the parasitic forms the study of free-living Protozoa should not be neglected." The parasitic forms are not considered degenerate but specialized by modifications that fit them for their peculiar environment. Several new or little known terms are introduced by the author: axoneme is used to describe the axial filament of a flagellum which, on leaving the body, is surrounded by a cytoplasmic sheath and becomes a flagellum; kinetoplast is employed for the combined parabasal and blepharoplastic complex; and gametocyst is the cyst wall formed around two gametocytes. The term neuromotor system used by Kofoid and his students to designate the karyosome, centrosomes, blepharoplasts, flagella, etc., is criticized, since some of the fibers included have a motor function but others are merely supporting rods without evidence that they are comparable to nerve fibrils, as the term suggests. The statement that only encysted intestinal amebas are able to carry infection to a new host since trophozoites "would, in all probability, be killed by the digestive fluids of the stomach," seems doubtful since the writer has shown that trophozoites can successfully withstand conditions in the stomach of lower animals and probably also in man.

Part 2, Systematic Description of the Protozoa with Special Reference to Parasitic and Coprozoic Forms, comprises 1,075 pages. This begins with an outline of the phylum (5 pp.), including brief descriptions of the larger divisions. Two subphyla are recognized, *Plasmodroma*, containing the classes *Rhizopoda*, *Mastigophora*, *Cnidosporidia* and *Sporozoa*, and *Ciliophora*, containing the classes *Opalinata*, *Ciliata* and *Suctorina*.

The class *Rhizopoda* (108 pp.) is introduced by a general statement (5 pp.) and a brief description of five orders (8 pp.), followed by a systematic description of the order *Amoebida* (95 pp.). This is largely devoted to the parasitic amebas that live in man, and species of the same genera living in other animals (77 pp.). Under each human species are given the chief synonyms, a brief historical account, the life history, morphology, pathogenicity, susceptibility of animals, cultivation and aberrant forms. In addition, paragraphs are inserted in appropriate places on the amebas of plants, the diagnosis of intestinal amebas of man, action of drugs and coprozoic amebas. One of the greatest services done by the author is to examine carefully the status of various species (?) of protozoa that have been described from man, and to express an opinion as to their validity. Thus, slides were studies on which there were specimens named by Chatterjee, *Entamoeba paradysenterica*; these Wenyon says are undoubtedly degenerate forms of *Entamoeba histolytica*. The author decides that enough evidence has not been advanced to prove that *Caudamoeba sinensis* and *Karyamoebina falcata* are not really *Entamoeba histolytica*. Regarding the theory of Kofoed, that one type of arthritis deformans is due to *E. histolytica*, Wenyon decides that more convincing evidence is required before this can be accepted. Other interesting decisions of the author are that the generic term *Entamoeba* should be employed for human species, and *Endamoeba* for the large ameba of the cockroach; that there is not any definite proof of emetine resistant strains of *E. histolytica*; that the characters that distinguish *Councilmania lafleuri* from *Entamoeba coli* fall within the range of variation of the latter; that *Entamoeba dispar* cannot be accepted as a "good" species on the basis of its being non-pathogenic since he does not believe "physiological data of this kind afford a means of distinguishing species"; that *E. sinensis* is probably not a distinct species; and that *E. bengalensis* may be *E. histolytica*. Hegner and Taliaferro (1924) are given credit for naming the ameba from the turtle, *Endamoeba barreti*, whereas this was done by Taliaferro and Holmes (1924).

The section on the class *Mastigophora* (448 pp.) is of greater length than that on the class *Rhizopoda*, largely because of the long description of the family *Trypanosomidae* (295 pp.). Special sections are interpolated on coprozoic *Mastigophora*, invasion of the blood stream by intestinal *Mastigophora*, blackhead in turkeys, inoculation of insect *Trypanosomidae* into vertebrates and invertebrates, and trypanosomes as filter passers. Various conclusions reached by the author regarding the *Mastigophora* are that such a genus as *Craigia* does not exist, based on the examination of poorly stained slides sent to Wenyon by Craig; that *Embadomonas bengalensis* of Chatterjee is probably *E. intestinalis*, and that *E. sinensis* of Faust and Wassell is a doubtful species; that the correct specific name of the human intestinal *Trichomonas* is *T. elongata*, Steinberg, 1862, and not *T. hominis* as now generally accepted; that the trichomonads of the human vagina, mouth and intestine belong to one species; that the two species of trichomonads, *T. muris* and *T. parva*, described by Wenrich in the rat cannot be accepted without further information; that the *Hexamita* described from man by Chalmers and Pekkola, slides containing which were examined by Wenyon, is really *Trichomonas*; that the so-called axostyles of *Giardia* are the axonemes of the posterior flagella; and that the cysts of *Giardia* may hatch within the same host in which they are formed since trophozoites are rarely found in division. Credit is given to Reichenow (1923) on page 627 for diagnosing *Chilomastix mesnili* by the culture method, whereas this was first reported by Hegner and Becker (1922) and confirmed by Reichenow. Trichomonads are said to be probably the commonest intestinal flagellates of man although surveys record a higher incidence

for both *Chilomastix* and *Giardia*. On page 663, the reader is referred to fig. 173, 4 for an illustration of *Sphaerita*, but no such organism appears in this figure. Doubt is cast on the phenomenon of multiple fission in trichomonads, but this undoubtedly occurs in *T. termopsisidis*, as described by Andrews (1925).

The class *Cnidosporidia* (44 pp.) is made up of the *Myxosporidiida* and *Microsporidiida*. Following a description of the general organization of the class there are detailed accounts of certain genera and species, especially the *Microsporidia* of blood-sucking *Arthropoda* and *Nematoda*, and the supposed *Microsporidia* in rabies and encephalitis in rabbits. The *Sarcosporidia*, *Haplosporidia* and *Rhinosporidium* (19 pp.), which are often included under the *Neosporidia*, are here treated as parasites of undetermined position.

Volume II begins with the class *Sporozoa* to which 374 pages are devoted. Two subclasses are recognized, the *Coccidiomorpha* and the *Gregarina*. A group of parasites of doubtful nature (12 pp.), such as *Toxoplasma* and *Cytamoeba*, and a list of structures of doubtful nature (13 pp.), such as *Anaplasma* and *Rickettsia*, are included. Special attention is given to the coccidia of the genera *Isospora* and *Eimeria* in man and other animals (57 pp.), and the commonest human form is referred to the species *Isospora belli*. Other groups to which considerable space is devoted are the *Plasmodiidae* (78 pp.), *Babesiidae* (37 pp.) and *Haemogregarines* (16 pp.). Wenyon accepts the work of Thomson and Robertson as proof that *Eimeria wenyoni* and *Eimeria oxyspora* are really coccidia of fish that are ingested occasionally by man and are later found in the feces. He believes the "black spores" sometimes found in the stomach wall of mosquitoes infected with malaria are not *Microsporidia* but the contents of degenerated oocysts, and that the *Haemogregarines* (?) described from man are "vegetable cells of extraneous origin." The author says that the period of growth of the bird malaria parasite is in doubt; he has evidently overlooked the convincing work of Mrs. Taliaferro (1925) in which she proves that a definite asexual cycle exists as in human malaria—in one strain, twenty-four hours, and in another, thirty hours.

The subphylum *Ciliophora* (77 pp.) is divided into the *Protociliata*, containing the class *Opalinata*, and the *Euciliata*, comprising the class *Ciliata*. The genera *Nyctotherus* and *Balantidium* in man and other animals are emphasized (14 pp.).

Part 3 is devoted to the spirochetes (56 pp.). A general account of their structure, affinities and nomenclature (4 pp.) is followed by a section on free-living species (3 pp.) and a longer discussion of parasitic species (49 pp.), divided into those living chiefly in the blood (24 pp.), in tissues (5 pp.) and in the alimentary, respiratory and genito-urinary tracts (13 pp.), and the parasitic spirilla (3 pp.).

Part 4 is entitled *Methods of Investigation and Rules of Nomenclature* (59 pp.). Here are discussed living protozoa, culture methods, maintenance in laboratory animals, killing fixing and staining and the dissection of mosquitoes and tsetse flies for detection of infection. Five pages are devoted to spirochetes. The inclusion of the International "Rules of Zoological Nomenclature reproduced from the Proceedings of the Ninth International Congress of Zoology," 1913 (14 pp.), will be appreciated by every protozoologist.

Part 5 lists the vertebrate hosts of blood parasites (51 pp.), and the invertebrate hosts of the *Trypanosomidae* (11 pp.).

Part 6 is a list of references to literature on the protozoa (89 pp.) and spirochetes (9 pp.). In all about 3,000 titles are given, forming a comprehensive bibliography of the subject. The list appears to be remarkably free

from errors, but it would be of even greater value if the length of the papers listed was indicated by page numbers.

An excellent index of forty-six pages completes the book. An authors index would have added materially to its value.

The book is printed on heavy glazed paper and is well bound. The volumes are large, but not too large to handle easily. There are 565 illustrations, well selected and uniformly excellent, and twenty colored plates, principally of blood-inhabiting species.

Dr. Wenyon has put an immense amount of time and effort into the writing of these volumes and has produced the best book on protozoology available in any language. It will no doubt be the standard reference book on the parasitic groups of protozoa for many years, and the author deserves the thanks of every student of this phylum for his masterly presentation of a complicated and difficult subject.

LYSSA BEI MENSCH UND TIER. By PROF. DR. R. KRAUS, Director des staatlichen serotherapeutischen Institutes in Wien; DR. MED. VET. F. GERLACH, Direktor der staatlichen Tierimpfstoffgewinnungsanstalt in Mödling bei Wien; DR. F. SCHWEINBURG, an der staatlichen Schutzimpfungsanstalt gegen Wut in Wien. Mit 69 Abbildungen im Text und 6 farbigen Tafeln. Berlin und Wien: Urban & Schwarzenberg, 1926.

This work of 464 pages is a complete treatise on the subject of rabies, and constitutes the first of a series of monographs to be prepared by Kraus on diseases caused by filtrable viruses.

It is more than a mere compilation of the literature, in that it includes the combined knowledge of the authors, all of whom have had years of laboratory and clinical experience with rabies. The authors acknowledge their indebtedness and free use of the older monographs of Högyes, Babes, Heller, Marie, Stimson and Aujeszky, and also the works edited by Kolle and Wassermann, Kraus and Levaditi, and in addition they have covered nearly all the literature of the last fifteen years.

The chapters on etiology, postvaccinal paralyses and protective vaccination of man and animals are specially thorough, and these are the subjects which hold particular interest today.

Concerning the nature of the virus great progress has not been made. In connection with their discussion of filtrability the authors refer to the discovery by Ricketts, in 1909, of cell inclusions in Rocky Mountain spotted fever and typhus. They also state that Noguchi's reported cultivation of rabies virus (1913) remains unconfirmed. They give a full account of fixed and street viruses, and the manner in which they behave in various hosts, and point out that according to several reputable authorities there appears to be more than one strain of street virus, and the corresponding fixed virus.

The chapter on postvaccinal paralysis gives all available statistical data, the types of reaction with prognosis and treatment. The etiology is discussed at length, and while the authors do not reach a definite conclusion, they appear to favor the view that brain tissue per se is an important factor. This view has some experimental foundation, and in addition, such reactions have been rarer in those clinics using a vaccine which contains a minimal amount of nervous tissue, or requires a smaller number of injections. The authors note particularly that vaccines prepared by the ether methods cause fewer paralyses. The method used in Vienna in severe cases seems worthy of particular mention. Fixed virus brain after being treated with ether for seventy-two hours is

administered in 10 cc. doses of a 10 per cent emulsion, daily, for the first six days, and this is followed by the usual Högyes dilution method. Death from rabies has not occurred in a series of about 800 cases thus treated; all wounds were severe, including many head bites by proved rabid dogs, and postvaccinal paralyses have not occurred.

Regarding protective vaccination in man, the authors regard vaccine prepared by one of the ether methods or a dilution method, or, in other words, a fairly active virus, as more potent in preventing rabies and also less apt to cause paralysis. The present tendency is to give a more active virus and a smaller number of doses.

The question of protective vaccination in dogs and other animals is discussed in full, particular emphasis being given the Japanese method and various modifications of the same, with the results obtained in various countries to date.

In general this book will be of particular value to libraries and to students of the rabies problem, in that every phase of the subject is covered. The chapters on the clinical course of the disease in man, and in all species of domestic and some wild animals, are exhaustive. The work of all important investigators of rabies is given in sufficient detail to make reference to the original work almost unnecessary.

The general criticism may be made that methods and results obtained in the United States are not given sufficient consideration, but this is probably due to the lack of concise and full statistics on the subject.

ENZYMES, PROPERTIES, DISTRIBUTION, METHODS AND APPLICATIONS. By SELMAN

A. WAKSMAN, M.S., PH.D., Associate Professor of Soil Microbiology, Rutgers University; Microbiologist, N. J. Agricultural Experiment Station, and WILBURT C. DAVISON, M.A., M.D., Associate Professor of Pediatrics, Johns Hopkins University, School of Medicine; Associate Pediatrician, Johns Hopkins Hospital. Pp. 364. Price, \$5.50. Baltimore: the Williams & Wilkins Co., 1926.

This monograph probably contains as much information about enzymes as can be packed into 270 pages of text. Actually, it is a reading index to a bibliography of 1,323 titles that occupies another eighty pages. A work that contains such a large amount of condensed information touching on all known features of enzymes and enzyme action, and leading the reader to the original sources, is certain to be useful to a wide group of laboratory workers, including medical men. This same condensation prevents any exhaustive, critical, or lucid discussion of many of the topics considered—for these the reader will generally have to consult the original literature, and particularly the numerous critical reviews cited in the bibliography. Certainly this is true in respect to the items of particular interest to pathologists. The discussion of autolysis fails to suggest the large amount of work done and information secured concerning autolysis as a factor in disease. The detailed information available concerning the elaborate series of enzymes participating in purine metabolism, and their peculiar distribution in different organs of different species, is not apparent from this text. The paragraph entitled "Enzymes as Antigens" (p. 93), does not discuss this subject, but is devoted principally to the toxicity of enzymes. There is no systematic consideration of the topic of Enzymes as Antigens, which, perhaps is not a great loss in view of the uncritical character of most of the published work. One notes the absence in bibliography and text to any reference to the contributions of Hopkins on oxidation. Sumner's work on the nature of urease is perhaps too recent for inclusion.

In general the impression is gained that in discussing the enzymes in pathologic conditions, there is too ready acceptance of some of the statements in the medical literature. It is perhaps not too much to say that in the matter of studies of enzymes in pathologic processes medical literature offers a larger proportion of chaff to wheat than in almost any other department, through the rushing in of physicians where chemists fear to tread. Experimental results have been presented and conclusions drawn, in all honesty, by novices lacking a full appreciation of the sources of error, or the limits of their methods and materials. Such literature needs highly sceptical consideration.

The task of reviewing all the literature dealing with enzymes and their activities is of hopeless magnitude, and one who calls attention to omissions or shortcomings in the outcome of such an enterprise must recognize and accept their unavoidability in a work of this kind. It is fair to say that the authors have done a creditable and highly useful task in their attempt "to piece these irregular and loosely fitting fragments together into a mosaic upon which future studies may rest."

THE MEDICAL DEPARTMENT OF THE UNITED STATES IN THE WORLD WAR:
VOLUME XIV. MEDICAL ASPECTS OF GAS WARFARE. PREPARED UNDER THE
DIRECTION OF MAJ. GEN. M. W. IRELAND, THE SURGEON GENERAL. BY
COL. WILDER D. BANCROFT, C.W.S., MAJOR H. C. BRADLEY, C.W.S., MAJOR
J. A. E. EYSTER, M.C., COL. H. L. GILCHRIST, M.C., CAPT. SAMUEL GOLD-
SCHMIDT, C.W.S., CAPT. PAUL J. HANSLIK, M.C., A. S. LOVENHART, M.D.,
ALFRED S. WARTHIN, M.D., CAPT. E. K. MATSHALL, JR., C.W.S., MAJOR
WALTER J. MEEK, C.W.S., MAJOR JAMES E. POORE, M.C., MAJOR A. M.
PAPPENHEIMER, M.C., TORALD SOLLMAN, M.D., SERGT. JESSE TARR, C.W.S.,
LT. COL. F. P. UNDERHILL, C.W.S., and CAPT. D. W. WILSON, C.W.S.
Washington: Government Printing Office, 1926.

The accounts of gross and microscopic changes in the bodies of 107 persons who died from poisonous gases, or from other causes subsequent to "gassing," together with the clinical details, constitute the most valuable portion of this volume. In some instances, the nature of the gas was not learned, but nearly all the changes described were attributed to mustard gas. From the late occurrence of many deaths, sometimes weeks after exposure to the gas, opportunity was afforded to study sequences. This section, dealing with the effects of gases used in warfare on the human body, is elaborately illustrated with colored plates and splendid drawings. In the legend for plate 9 the word "Branches" appears where Bronchus is meant.

The largest part of the volume is a reprinting of monographs and articles published in journals on the experimental work with animals and on other studies carried out when information about poisonous gases became imperative. To have the results of such investigations thus made accessible in a single volume will prove highly useful.

Books Received

MUSCULAR CONTRACTION AND THE REFLEX CONTROL OF MOVEMENT. By J. F. Fulton. Pp. 644. Price, \$10. Baltimore: Williams & Wilkins Company, 1926.

SYMBIONTICISM AND THE ORIGIN OF SPECIES. By Ivan E. Wallin, Professor of Anatomy, School of Medicine, University of Colorado. Pp. 171. Price, \$3. Baltimore: Williams & Wilkins Company, 1927.

THE DE LAMAR LECTURES 1925-1926 OF THE SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY. Edited, with an introduction by W. H. Howell. Pp. 220. Price, \$5. Baltimore: Williams & Wilkins Company, 1927.

Contains lectures as follows: David Marine, Prevention of Simple Goiter; Alice Hamilton, Industrial Toxicology in U. S.; Francis Carter Wood, Necessity of Education in Control of Cancer; Frederick W. Sears, Problems in Rural Hygiene; Thomas Adams, Industrial Housing and Public Health; W. G. Smillie, Intensity Surveys of Hookworm Infestation; Edw. R. Baldwin, Absolute and Relative Value of Laboratory Procedures in the Diagnosis of Tuberculosis; Louis I. Dublin, Body Build and Longevity; Joseph Goldberger, Etiology of Pellagra; C. R. Stockard, Constitutional Types in Relation to Disease; Horace Lo Grasso, Heliotherapy in Tuberculosis; Robbins B. Stoeckel, Motor Vehicle Accident Prevention; Sir Arthur Newsholme, William Fari the Father of English Vital Statistics.

A SURVEY OF AMERICAN CHEMISTRY, Vol. 1, July 1, 1925, to July 1, 1926. Including Reports from Scientific Committees, Division of Chemistry and Chemical Technology, National Research Council. Edited by William J. Hale, Chairman of the Division, in cooperation with Clarence J. West, Chairman, Committee on Publications and Publicity, National Research Council. Contributors: Roger Adams, Edward Bartow William H. Bassett, Marston T. Bogert, Elmer K. Bolton, Herbert M. Boylston, Colin G. Fink, Francis C. Frary, Charles L. Gabriel, John A. Gann, William C. Geer, Harry LeB. Gray, William J. Hale, Emmet F. Hitch, Claude S. Hudson, Walter R. Ingalls, Treat B. Johnson, Charles A. Kraus, Phoebus A. Levene, Samuel C. Lind, Paul D. Merica, Harlan S. Miner, Charles E. Munroe, Lester A. Pratt, E. Emmet Reid, Francis O. Taylor, Hugh S. Taylor, Gustave W. Thompson, Donald D. Van Slyke, Harry B. Weiser, Frank C. Whitmore, James R. Withrow. Pp. 257. Price, \$2. New York: The Chemical Catalog Company, Inc., 1927.

DIE MORPHOLOGIE DER MISSBILDUNGEN DES MENSCHEN UND DER TIERE. Ein Hand- und Lehrbuch für Morphologen, Physiologen, Praktische Aerzte und Studierende, unter Mitwirkung zahlreicher Fachgenossen, begründet von Weil and Prof. Dr. Ernst Schwalbe. Herausgegeben von Dr. G. B. Gruber, O. Oe. Professor der Pathologischen Anatomie an der Universität Innsbruck. II. Teil: Die Einzelmisbildungen XII. Lieferung, 3. Abteilung, 1.-3.

576 *ARCHIVES OF PATHOLOGY AND LABORATORY MEDICINE*

Kapitel: Die Missbildungen des Muskelsystems (ausschliesslich des Zwerchfells) von Dr. A. Binder, Pforzheim. Die Missbildungen des Zwerchfells, von Prof. Dr. G. B. Gruber, Innsbruck. Die Missbildungen der Harnorgane, von Prof. Dr. G. B. Gruber, Innsbruck, mit einem Bildniss und einem Einleitenden Gedenkwort an Ernst Schwalbe von Geh. Prof. Dr. Paul Ernst, Heidelberg, mit 1 Titelbild und 252 Abbildungen im Text. Pp. 374. Paper. Price, 20 marks. Jena: Gustav Fischer, 1927.

IMMUNITY IN SYPHILIS. By Alan M. Chesney, Johns Hopkins Medical School. Pp. 85. Price, \$2.50. Baltimore: Williams & Wilkins Company, 1927.

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